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DEPARTMENT OF MEDICINE, HUDDINGE  
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# **CERVICAL INTRAEPITHELIAL NEOPLASIA IN MIGRANT WOMEN LIVING WITH HIV**

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# Cervical intraepithelial neoplasia in migrant women living with HIV

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

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For my parents Carin and Bosse



# ABSTRACT

The **aim** of this thesis was to study high-grade cervical intraepithelial neoplasia (CIN) in women living with HIV (WLWH) in Sweden compared with HIV-negative women (HNW) of the same region of birth.

In **Paper I** we assessed the cumulative incidence (CuI) and hazard ratio (HR) of CIN3, adenocarcinoma, and invasive cervical cancer (CIN3+) in a cohort of WLWH (n = 893) and HNW (n = 205 842) by linking the Swedish national HIV registry (InfCare HIV) with the Swedish Population Registry (SPR) and the Swedish National Cervical Screening Registry (NKCx). The CuI of CIN3+ was 13.1% (95% CI 8.9-17.2) and 2.1% (95% CI 2.0-2.2) for WLWH and HNW, respectively. WLWH had more than eight times higher risk of CIN3+ than HNW (HR 8.8: 95% CI 6.9–11.3), increasing with the level of immunosuppression. The highest risk was seen in WLWH born in the East region, dominated by Thai women.

In **Paper II** we analysed if the prevalence of undiagnosed HIV in women diagnosed with CIN2+ (n = 62 874) in the counties of Stockholm and Gothenburg, Sweden, would reach the threshold of 0.1%, which has been suggested cost-effective for HIV-testing. The proportion of undiagnosed HIV was calculated by linking NKCx with InfCare HIV and did not exceed 0.1% in all women, indicating that HIV-testing all women with CIN2+ in Sweden may not be cost-effective. However the proportion of undiagnosed HIV exceeded 0.1% among migrant women diagnosed with CIN2+ suggesting that HIV-testing should routinely be performed in this population.

In **Paper III** we assessed outcome after treatment of CIN2+ in 140 WLWH and 284 HNW, matched for country of birth, identified by linking NKCx with InfCare HIV and SPR. WLWH were three times more likely to have treatment failure (odds ratio (OR) 3.7 [95% CI 2.0-6.8]) and five times more likely to recur (hazard ratio 5.0: 95% CI 2.1-11.6) than HNW. Suppressive (HIV-RNA<50 copies/mL) antiretroviral therapy (ART) at time of treatment of CIN2+ was associated with reduced odds ratio of treatment failure (OR 0.3: 95% CI 0.1-0.8).

In **Paper IV** we studied whether HPV genotypes in women with CIN2+, identified in paper III, differed depending on their HIV status. Cervical tissue blocks of included women were retrieved from bio banks and HPV type was identified using modified general primer PCR and Luminex genotyping. WLWH were less likely to be infected with HPV 16 (prevalence ratio [PR] 0.6: 95% CI 0.35-0.97), and more likely to be infected with multiple high-risk (HR) HPV (PR 2.1, 95% CI 1.17-3.79). Only 25% of WLWH vs. 47% of HNW had HR HPV types that are covered by the bivalent and quadrivalent HPV vaccines (HPV 16 and/or 18) (PR 0.6: 95% CI 0.38-0.97).

In summary, my thesis showed that WLWH in Sweden are at higher risk of developing CIN3+, have poorer outcome after treatment of CIN2+, and less proportion of HPV 16 than HNW. We also found level of immunosuppression and, for the first time, suppressive ART to be associated with effective treatment of CIN2+. We recommend migrants diagnosed with CIN2+ to be HIV-tested. Early HIV diagnosis, access and adherence to ART, HPV vaccination of young people living with HIV and those at high-risk of HIV-infection, and finally access and adherence to cervical cancer screening are all crucial to minimize the incidence of CIN2+ and its progression to ICC in WLWH.

## LIST OF SCIENTIFIC PAPERS

- I. Carlander C, Wagner P, Svedhem V, Elfgren K, Westling K, Sönnernborg A, Sparén P. **Impact of immunosuppression and region of birth on risk of cervical intraepithelial neoplasia among migrants living with HIV in Sweden.** Int J Cancer. 2016 Oct 1; 139 (7): 1471-9
- II. Carlander C, Marrone G, Brännström J, Yilmaz A, Elfgren K, Sparén P, Sönnernborg A. **Assessing cervical intraepithelial neoplasia as an indicator disease for HIV in a low endemic setting: a population-based register study.** BJOG. 2017 Oct; 124 (11): 1680-1687.
- III. Carlander C, Wagner P, van Beirs A, Yilmaz A, Elfgren K, Dillner J, Sönnernborg A, Sparén P. **Suppressive antiretroviral therapy associates with effective treatment of high-grade cervical intraepithelial neoplasia.** AIDS. 2018 Jul 17; 32 (11): 1475-1484.
- IV. Carlander C, Lagheden C, Eklund C, Nordqvist Kleppe S, Wagner P, Yilmaz A, Elfgren K, Sönnernborg A, Sparén P, Dillner J. **HPV genotypes in migrant women with high-grade cervical neoplasia differ depending on HIV status; an interim analysis of a population-based register study.** In manuscript.

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## LIST OF ABBREVIATIONS

ADC	AIDS defining cancer
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
CD4-cells	CD4 <sup>+</sup> T-lymphocytes
CD4 count	CD4 <sup>+</sup> T-lymphocytes/ $\mu$ l serum
CIN	Cervical intraepithelial neoplasia
CIN2+	CIN grade 2, grade 3, adenocarcinoma in situ, cervical cancer
CIN3+	CIN grade 3, adenocarcinoma in situ, cervical cancer
EEA	European Economic Area
EU	European Union
HIV	Human Immunodeficiency Virus
High-grade CIN	Cervical intraepithelial neoplasia grade 2 and 3
HNW	HIV-negative women
HPV	Human Papilloma Virus
HR HPV	High-risk HPV (carcinogenic)
HSIL	High-grade squamous intraepithelial neoplasia
ICC	Invasive cervical cancer
InfCare HIV	Swedish National HIV Registry
IVDU	Intravenous drug use
Late presenter	CD4 count <350 and /or AIDS at time of HIV diagnosis
LMIC	Low and Middle Income Countries
LSIL	Low-grade squamous intraepithelial lesion
Non-HPV 16/18	Other HPV genotypes than 16 or 18
NKCx	Swedish National Cervical Screening Registry
PLWH	People living with HIV
pRB	Retinoblastoma tumour suppressive protein
SSA	sub-Saharan Africa
SPR	Swedish Population Registry
Suppressive ART	HIV-RNA<50 copies/mL (in this thesis)
WHO	World Health Organization
WLWH	Women living with HIV

# 1 PREFACE

As much as I enjoy being an infectious disease clinician, I must admit that nothing beats being an HIV-doctor. When I got the opportunity to combine my clinical work with research it was very important to me that I would be able to see a connection between those two; my clinical work and my research. Also, I wished that, if possible, both my own patients and those living with HIV in low and middle-income countries would benefit from our research. While most research on HIV is still based on white American males, most of my patients are migrant women born in sub-Saharan Africa and Thailand and it was with great satisfaction that I joined a project focusing on these women.

I think that the true joy that working on this thesis has brought me is because I can see my patients (anonymously) in every data file, every hazard ratio and every conclusion. It is all that I can wish for that these findings might also be a small brick in the building of a healthier population living with HIV.





## 2 INTRODUCTION

As an increasing number of people living with human immunodeficiency virus (HIV) worldwide are receiving antiretroviral therapy (ART), new problems arise. While access to ART has decreased the mortality of acquired immune deficiency syndrome (AIDS) and the overall life expectancy is getting closer to that of HIV-negative individuals, cancer development is now one of the major threats in this population<sup>1-5</sup>.

Persistent infection with carcinogenic human papillomavirus (HPV), so called high-risk HPV (HR HPV), is a necessary cause of cervical intraepithelial neoplasia (CIN), which may in turn progress to invasive cervical cancer (ICC). Due to HIV-induced immunosuppression, women living with HIV (WLWH) are more likely than HIV-negative women (HNW) to have persistent HR HPV cervical infections<sup>6,7</sup>. Consequently, there is an increased global burden of ICC, and its precursor high-grade CIN (CIN grade 2 and 3), in WLWH<sup>8-11</sup>.

The majority of WLWH diagnosed with HPV-related disease are currently living in low and middle income countries (LMIC) where access to cervical cancer screening is often poor<sup>11</sup>. ICC is the most common cancer among women in Eastern and Central Africa and if anything the numbers are rising<sup>12,13</sup>. At the time of the initiation of this thesis there was very little data concerning migrants living with HIV who had moved from regions such as sub-Saharan Africa (SSA), with the highest incidence of cervical cancer in the world, to a European setting with access to better healthcare. There were many unanswered questions such as: Was the risk of cervical precancerous lesions higher among migrants living with HIV compared to WLWH born in Sweden? Or compared to HNW migrating from the same region? And how was their prognosis after treatment of high-grade CIN? Do migrants living with HIV share the same HPV-genotypes, which have implications for HPV vaccination and HPV triaging, as Swedish-born WLWH or rather the same as HNW from the same region of birth? The studies of this thesis focus on these questions.



## 3 BACKGROUND

### 3.1 HIV

HIV is a lentivirus within the *Retroviridae* family that is composed of two copies of a single-stranded RNA and surrounded by a lipid bilayer membrane derived from the infected human host cell<sup>14</sup>. Two different HIV types, HIV-1 and HIV-2, can infect humans of which the latter is mainly seen in a few West African countries, France, Portugal and former Portuguese colonies. HIV-2 is therefore not the focus of this thesis. HIV-1 is phylogenetically classified into several groups, where the M (major) group, representing >90% of all HIV infections, can be further subdivided into nine subtypes (A-D, F-H, J, K) and a larger number of circulating recombinant forms<sup>15</sup>. Subtype C predominates in sub-Saharan Africa and India while subtype B until now has dominated in Western Europe and the US, but with migration this distribution is changing<sup>16</sup>.

The virus infects host cells, most importantly CD4+ T-lymphocytes (CD4-cells), by attaching to the CD4 receptor and one of the co-receptors CCR5 or CXCR4 (Figure 1). Through the use of the viral enzymes reverse transcriptase and integrase, double-stranded DNA is generated from the viral RNA templates and integrated into the human genome, thus establishing a viral reservoir. A high replication rate, together with the fact that the enzyme reverse transcriptase is prone to making mistakes, a large viral load with great diversity is developed<sup>17</sup>.

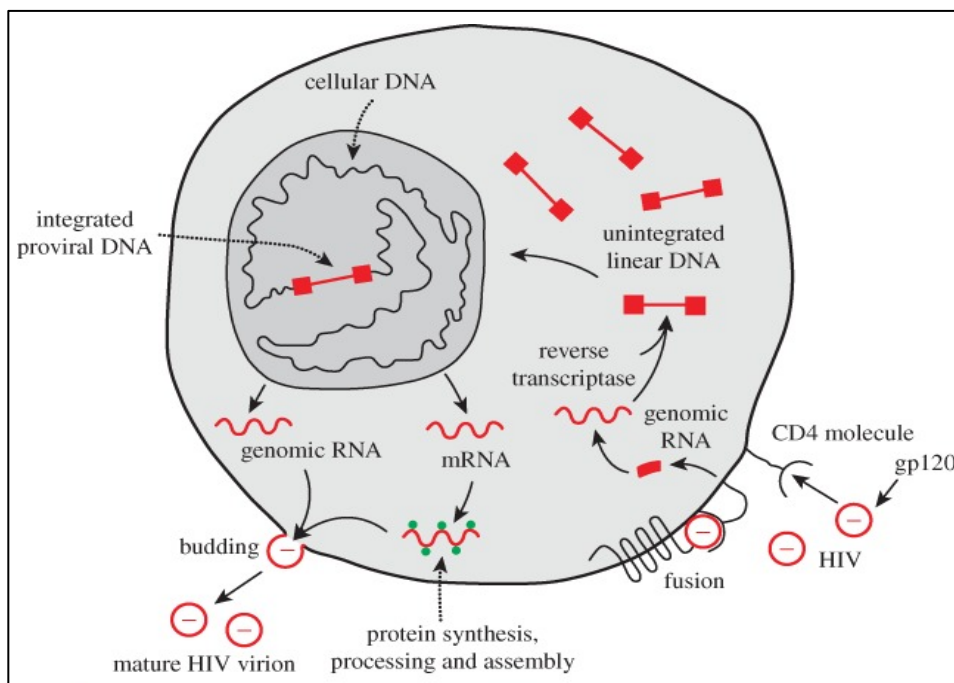
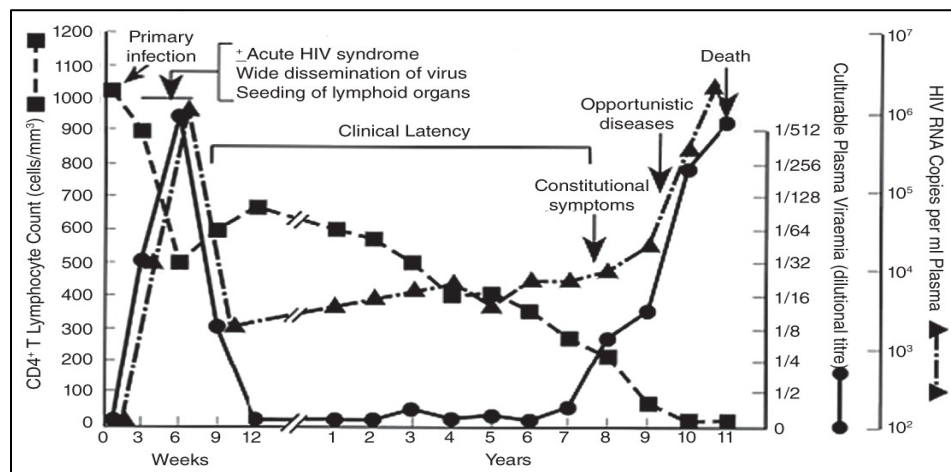


Figure 1. Life cycle of HIV<sup>18</sup>.

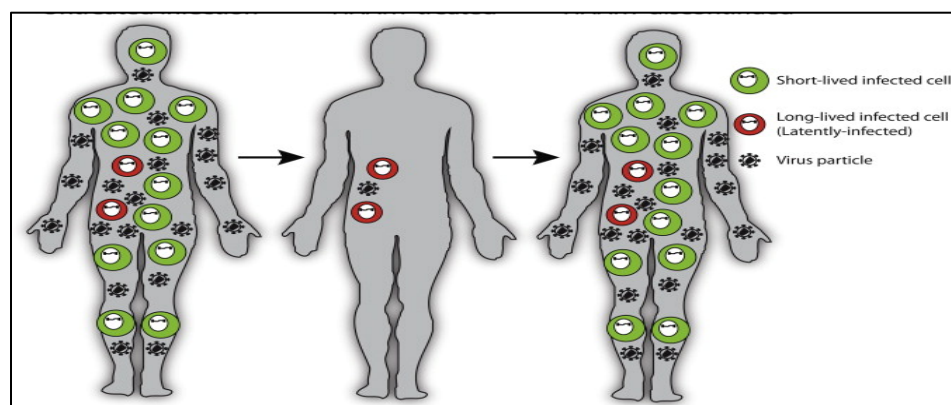
Within one to four weeks after infection a (most often) flu-like illness may appear, known as primary HIV infection, which may last up to a month. Viral replication peaks about one

month after infection and is followed by a period of asymptomatic chronic infection with lower viral replication but with a progressive loss of CD4-cells (Figure 2). If an HIV-infected individual is not treated with ART the CD4-cells will continue to decrease, leading to immunosuppression. Typically, HIV-associated diseases, such as oral candidiasis or herpes zoster, will appear once the CD4<sup>+</sup> T-lymphocytes/ $\mu$ l (CD4 count) is below 350 (normal range 490-1340). AIDS defining conditions, such as tuberculosis and pneumocystis pneumonia, may also appear, especially when the CD4 count is below 200. On average it takes 8-10 years from infection to AIDS in untreated individuals<sup>19</sup>.



**Figure 2. Time course of HIV infection in an untreated individual<sup>18</sup>.**

Once an HIV-infected individual has started adequate ART, the HIV replication is suppressed and the plasma viral load decreases to concentrations below the lower limit of detection (i.e. suppressive ART, defined as < 50 or <20 HIV RNA copies/mL plasma, depending on the method used), in most people within six months (Figure 3). This leads to a halt of further destruction of the immune system, although a chronic immune activation and inflammation remains (more on that below). Some people, especially those who start ART at very low CD4 counts, have an impaired CD4 T-cell recovery despite virological suppression. This is associated with an increased risk of non-AIDS-related morbidity and mortality<sup>20,21</sup>.



**Figure 3. Individual with untreated HIV (>10<sup>9</sup> infected cells and virions produced and cleared every day), ART-treated HIV (low, often undetectable, viral loads but HIV persists in latently infected cells) and ART-discontinued HIV<sup>22</sup>.**

The increasing availability of ART has greatly reduced mortality and morbidity in people living with HIV (PLWH) and life expectancy is getting closer to that of the HIV-negative population, especially if HIV diagnosis is early<sup>23-25</sup>. In addition, PLWH who are on suppressive ART with undetectable virus in plasma do not transmit the virus<sup>26,27</sup>.

### **3.1.1 HIV-ASSOCIATED IMMUNE ACTIVATION AND INFLAMMATION**

HIV-infection is associated with activation of both the innate and adaptive parts of the immune system. At the time of HIV-transmission an extensive systemic immune activation is initiated, never to subside completely and even patients with suppressive ART are found to have a remaining chronic immune activation and thus low-grade inflammation<sup>28</sup>.

The depletion of CD4-cells is partly due to apoptosis of HIV-infected cells, but the majority of CD4-cells die of caspase-1 mediated programmed cell death (pyroptosis). While pyroptosis may be effective in an acute bacterial infection, it unfortunately has a detrimental effect in the pathology of HIV-infection. The release of pro-inflammatory cytokines secondary to pyroptosis creates a pathogenic vicious cycle in which dying CD4-cells release inflammatory signals that attract more cells into the infected lymphoid tissues with increased chronic inflammation and tissue injury as a consequence<sup>29</sup>.

Microbial translocation through the gut-blood barrier has been found to be an important inducer of HIV-associated immune activation. It is caused by damage to the mucosal gut barrier which starts already at time of primary HIV-infection due to depletion of CD4-cells from gut associated lymphoid tissue. Translocation of bacterial products across the damaged gut barrier activates macrophages and dendritic cells to produce pro-inflammatory cytokines that keeps the immune system activated<sup>30</sup>. The chronic inflammation thus seen even in patients with suppressive ART, which can be measured by increased levels of pro-inflammatory biomarkers such as CRP, IL-1, IL-6, and TNF- $\alpha$ , is considered to be associated with an increased risk of cardiovascular, neurocognitive and not the least malignant disease<sup>31</sup>.

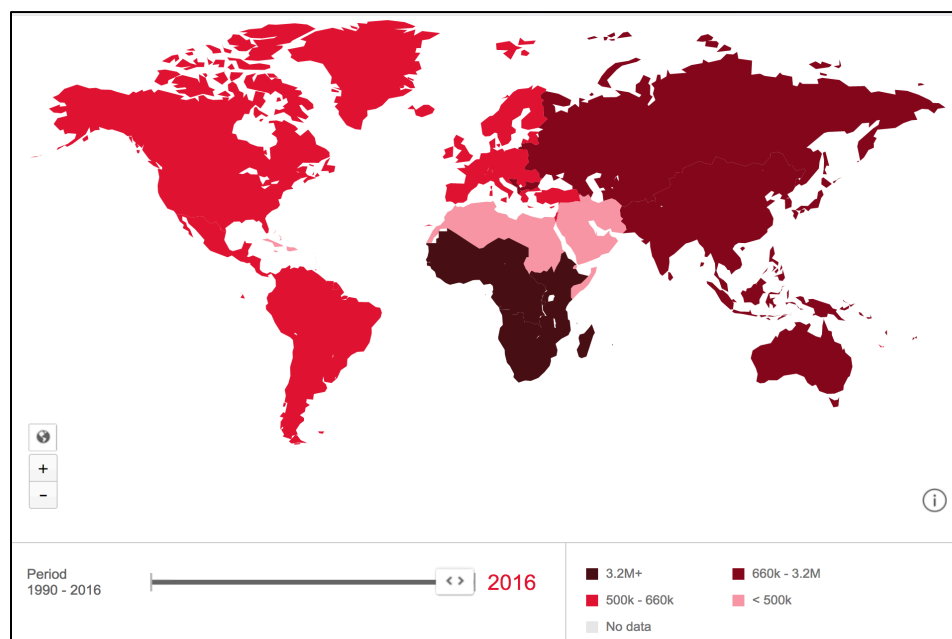
### **3.1.2 HIV AND CANCER**

PLWH have an increased risk for many cancers compared to the HIV-negative population, most importantly virus-related cancer<sup>32,33</sup>. This is due to HIV-induced immunosuppression causing impaired control of oncogenic viruses such as HPV, Epstein-Barr-virus, Human-herpes-virus-8, Hepatitis C and Hepatitis B<sup>8</sup>. HIV-associated chronic inflammation and environmental/life-style factors may also influence the increased risk of cancer seen in PLWH<sup>34</sup>.

Cancers in PLWH are defined as AIDS-defining (ADC) (Kaposi sarcoma, non-Hodgkin's lymphoma and cervical cancer) and non-AIDS-defining (NADC) cancers. With access to suppressive ART and increasing longevity in PLWH the excess cancer burden has shifted from ADCs to NADCs<sup>3,4</sup>. While an impressive decline has been seen for Kaposi's sarcoma and non-Hodgkin's lymphoma the effect of ART on ICC has not been as clear (more on that below). Virus-related cancers dominate among NADCs together with lung cancer, the latter seems to be increased in PLWH even after adjusting for smoking<sup>32</sup>.

### 3.1.3 EPIDEMIOLOGY OF WOMEN LIVING WITH HIV

While the global prevalence of HIV is increasing because people on ART are living longer, the global incidence is decreasing. It is estimated that there are 36.7 million (30.8-42.9 million) PLWH in the world today of whom about half, 17.8 million (15.4-20.3 million) are women (15+ years old) (Figure 4). Approximately 80% of WLWH reside in SSA, 10% in Asia and Pacific, 4% in Eastern Europe and Central Asia and 3% in Western and Central Europe and North America<sup>35</sup>.



**Figure 4. Global distribution of women living with HIV (prevalence)<sup>36</sup>.**

Even though new HIV-infections have declined globally by 11% among adults (women and men) and by 47% among children (girls and boys) since 2010, young women remain at an unacceptably high risk of HIV in high-prevalence settings. Adolescent girls and young women (15–24 years old) accounted for 20% of new HIV-infections among adults worldwide in 2015, despite representing just 11% of the adult population<sup>35,37</sup>.

### 3.1.4 WOMEN LIVING WITH HIV IN SWEDEN

As of April 2018 there were 7536 individuals receiving care for HIV-infection in Sweden of whom 2926 (39%) were women. Of these women, 466 (16%) were born in Sweden and 2460 (84%) were migrants. Almost all women (96%) were on ART and of these practically all (97%) had suppressive ART at the time of the last measurement and a vast majority (87%) had a CD4 count >350, altogether indicating an exceptionally well-controlled HIV-cohort. In fact, Sweden was the first country to reach the 90-90-90 goal of UNAIDS; 90% of estimated HIV infected are diagnosed, 90% of diagnosed are on ART and 90% of those on ART are on suppressive treatment<sup>38,39</sup>.

Almost half (47%) of WLWH in Sweden have at one point been severely immunosuppressed with a CD4 nadir (lowest CD4 count ever measured for that individual) below 200<sup>40</sup>. Over the years the epidemiology of the female HIV-population in Sweden has changed with a decrease of those infected by intravenous drug use (IVDU) and an increase of heterosexually

infected migrants, in particular women born in SSA and South-East Asia<sup>41</sup>. Today 7% of WLWH in Sweden are presumed to be HIV-infected due to IVDU<sup>40</sup>.

### 3.1.5 LATE HIV PRESENTATION

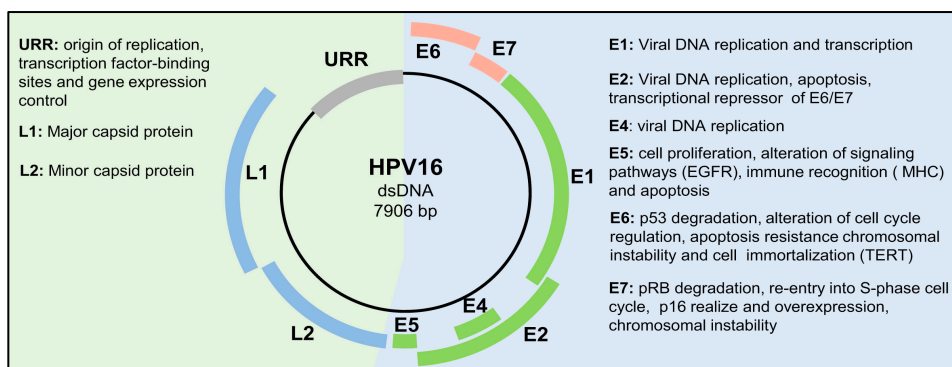
Of PLWH in the European Union and the European Economic Area (EU/EEA) one in seven are estimated to be unaware of their diagnosis and approximately half of all PLWH are diagnosed late (i.e. having a CD4 count below 350 cells/mm<sup>3</sup> or AIDS at time of HIV-diagnosis)<sup>42,43</sup>. A Swedish study from our research group found that more than half of newly diagnosed patients were late presenters and that this was associated with higher age and migrant status<sup>44</sup>. Similarly, being a female migrant from LMIC is associated with late presentation in the EU/EEA<sup>45</sup>. Late HIV-diagnosis has serious consequences and is associated with higher mortality, morbidity, high risk of HIV-transmission and increased healthcare costs<sup>42,46,47</sup>.

To prevent late HIV-diagnosis optimized HIV-testing is essential. Universal HIV-screening has been estimated to be cost-effective when the prevalence of undiagnosed HIV in a certain population reaches the threshold of 0.1%<sup>48-50</sup>. A European multicentre study found that eight diseases, including cervical or anal cancer/dysplasia, were associated with a prevalence of undiagnosed HIV above 0.1%. Consequently, HIV-testing was recommended for individuals presenting with these diseases, so-called indicator guided HIV-testing<sup>51-54</sup>.

Although HIV-testing is recommended when women with unknown HIV status are presenting with high-grade CIN, this seems not to be generally performed. At the time of the initiation of this thesis, the prevalence of undiagnosed HIV among women diagnosed with high-grade CIN in a low HIV-endemic setting, such as Sweden, was unknown.

## 3.2 HUMAN PAPILLOMAVIRUS

Human papilloma virus is a DNA virus from the papillomavirus family. In the human body the virus is most commonly found where there is a transition between squamous and glandular cells in the epithelium (squamocolumnar junctions) such as in the cervix, anus and tonsils<sup>55</sup>.



**Figure 5. HPV 16 structure and viral proteins<sup>56</sup>.**

HPV consists of circular double-stranded DNA surrounded by a protein shell composed of two proteins (L1 and L2). Only one strand of the double stranded DNA serves as a template for transcription. It contains three genomic regions; the early region (E) encodes viral



regulatory proteins, the late region (L) encodes the two capsid proteins and the upstream regulatory region (URR) contains the origin of the DNA replication and transcription control sequences (Figure 5)<sup>57</sup>.

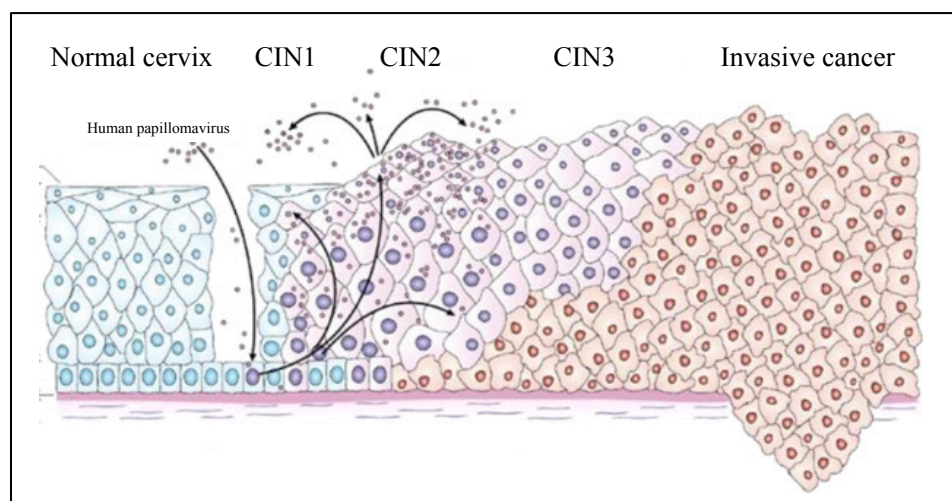
HPV is, contrary to HIV, genetically very stable. E6 and E7 are the primary HPV oncogenes due to their ability to inhibit essential steps in the human cell cycle. Among other cellular targets the oncogene E6 inhibits apoptosis through the inhibition of P53 while the oncogene E7 cancels cell-cycle arrest by inhibiting retinoblastoma tumour suppression protein (pRB). This may lead to genomic instability, loss of cell-growth control and eventually cancer development<sup>55,56</sup>.

Classification of HPVs is based on DNA sequence homology, more specifically on the nucleotide sequence homology of the L1 gene as this is the most conserved region of the viral genome. Within the family Papillomaviridae there are five different genera (alpha, beta, gamma, mu and nu) and to date 221 different HPVs have been classified (Figure 6)<sup>58,59</sup>.

Alpha papillomaviruses infect epithelial cells in genital mucosa and among these 13 genotypes constitute the HR HPVs; the carcinogenic group 1: HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and the probably carcinogenic group 2A: HPV 68. Nine HPVs are classified as possibly carcinogenic (group 2B: HPVs 26, 30, 53, 66, 67, 69, 70, 73, 82)<sup>60,61</sup>.

Benign HPV may cause genital condyloma, most commonly HPV 6 and 11. HR HPV types 16 and 18 are together estimated to cause around 80% of ICC worldwide<sup>60,62</sup> (Figure 7). In a large Swedish population-based study with 2850 cases of ICC the most common HR HPV type was HPV 16 (60%), followed by HPV 18 (19%), HPV 45 (7%) and HPV 31 (3%)<sup>63</sup>.

HPV is mainly transmitted during sexual activity and is the world's most common sexually transmitted infection with about 80% of the world's female population being infected at some point in life. An HPV infection is generally cleared within 12-24 months, but approximately 10% of women fail to clear the virus and the HPV infection becomes persistent<sup>64</sup>. In the cervix, HPV infects basal cells in the epithelium and it then uses the differentiation of the epithelium to move upwards, shedding virions at the cervical surface (Figure 6).



**Figure 6. HPV infection and different potential cervical intraepithelial lesions (adapted) <sup>56</sup>.**



Persistent infection of the cervical transformation zone with HR HPV is a necessary, but not sufficient, cause of ICC and its precursors CIN grade 1-3 (CIN3 equals cancer in situ). CIN is the term generally used for histology confirmed lesions while for cytology confirmed lesions the term generally used is squamous intraepithelial lesion (SIL) which is further divided into low-grade (LSIL) or high-grade (HSIL)<sup>56</sup>. In Sweden, the nomenclature has recently been changed, now using LSILcyt/HSILcyt and LSILhist/HSILhist, but for this thesis the term CIN is used for histology confirmed lesions<sup>65</sup>.

### **3.2.1 HIV AND HPV**

WLWH have higher prevalence and cumulative incidence of HPV and are more likely to have persistent HR HPV infections than HNW<sup>66-68</sup>. A global meta-analysis found much higher prevalence of HPV (41%) in normal cervical samples in WLWH than what was seen in similar samples of HNW (12%) in an earlier study<sup>69,70</sup>. HPV prevalence is highest in WLWH living in Africa and Latin America, but differences compared to other regions decrease with increasing severity of lesions<sup>69</sup>. In women diagnosed with CIN3/ICC, more than 90% are HR HPV-positive independent of HIV-status.

Although HIV and HPV are both sexually transmitted viruses studies, where lifetime sexual history was accounted for, have shown that the higher risk of persistent HR HPV-infection seen in WLWH compared with HNW is not only due to higher risk of exposure, such as number of sexual partners<sup>66,71,72</sup>. Rather, the difference seen between WLWH and HNW mainly seems to be due to HIV-induced immunosuppression, which is believed to cause less efficient HPV clearance and perhaps reactivation of latent HPV. Consequently, the prevalence and cumulative incidence of HR HPV increases with the level of HIV-induced immunosuppression, as expressed by a decreased CD4 count<sup>66,73-77</sup>. De Vuyst et al. were one of the first to show, even though some earlier studies had indicated the same, that ART use is associated with a lower prevalence of HR HPV compared to WLWH not on ART and this has later been confirmed in a meta-analysis<sup>78,79</sup>.

A number of studies have shown a broader range of HPV types in WLWH and these women have been found to have a lower relative prevalence of HPV 16 in normal cervical cytology<sup>69,73,80-82</sup>. It has been suggested that HPV 16 is the type which best evades a competent immune system, but that with increasing immunosuppression the fraction of non-HPV 16 genotypes (i.e. 18/31/33/35/39/45/51/52/56/58/59/68) increase among persistent HR HPV infections<sup>83</sup>. Whether this is true for normal/low-grade lesions only has been debated but later studies indicate that, as in HNW, genotypes 16, 18 and 45 are the most commonly detected in ICC in WLWH although the proportion caused by HPV 16 is significantly lower<sup>84</sup>.

Multiple HPV infections seem to result from independent events and have been suggested to be a biomarker for susceptibility to HPV infection<sup>85</sup>. WLWH are significantly more prone to have multiple HPV infections than HNW, increasing with the severity of immunosuppression but decreasing with severity of lesions<sup>73,86</sup>. The clinical significance of multiple infections is unclear as prospective evidence is limited<sup>86,87</sup>. Testing for HPV from biopsies rather than from cervical cytologies is known to reduce the prevalence of multiple infections, however,

previous studies of HPV types present in WLWH are, unlike data in this thesis, mostly from cervical cytologies<sup>69,84,88</sup>.

### **3.3 INVASIVE CERVICAL CANCER AND ITS PRECURSOR CIN**

As mentioned earlier, persistent HPV infections may lead to malignant transformation of the cervix uteri. The initial lesions, CIN1 and CIN2, may resolve spontaneously or progress to CIN3 (i.e. cancer in situ) and further to ICC. Typically, it takes 2-5 years from the detection of HR HPV to the development of CIN3 and it is estimated that about 30% of these progress to ICC within 10 years<sup>89</sup>. Known risk factors for the development of ICC are smoking, use of oral contraceptives, multi parity and immunosuppression<sup>56,90,91</sup>. Of the global burden of ICC almost all are squamous cell carcinomas (about 80%), and the remainder are adenocarcinomas (about 10%) or so called “rare types of ICC” (about 5%), but with some regional variability<sup>92</sup>.

ICC is the fourth most common cancer in women globally, the second most common in SSA (age-standardized incidence rates above 30/100 000) and the most common in women in Eastern and Middle Africa<sup>11,12</sup>. Annual incident cases worldwide are estimated to 528 000 cases (85% in LMIC) and 266 000 deaths (nine in ten in LMIC) worldwide<sup>12</sup>.

#### **3.3.1 ICC AND CIN IN WOMEN LIVING WITH HIV**

WLWH have a substantially increased risk of developing CIN2/CIN3 and ICC compared to HNW<sup>8-10,93</sup>. Although ICC was classified as an AIDS-defining disease as early as in 1993, it was not until much later it was actually proven to be more common in WLWH than in the HIV-negative population<sup>1,9,94-98</sup>.

In WLWH, similar to HNW, persistent HR HPV infection is essential for this development and consequently WLWH who are HR HPV negative with normal cytological findings have similar low risk of developing CIN2+/CIN3+ as do HNW<sup>99</sup>. On the other hand, WLWH who test positive for HPV 16 despite a normal cervical cytology have a higher risk of developing CIN2+/CIN3+ than do HNW, especially those immunosuppressed at the time of testing HPV 16 positive<sup>100</sup>.

The main risk factor for the development of CIN and ICC in WLWH is immunosuppression<sup>1,101</sup>. A low CD4 nadir or low current CD4 count are both associated with an increased risk of CIN2+<sup>79,102-104</sup>. If this is mainly due to the reduced ability of the immunosuppressed woman to clear HPV or if the oncogenic process is enhanced in other ways is unclear. An increased risk of CIN2+/ICC is also seen in women who are immunosuppressed for other reasons than HIV, for example solid transplant recipients<sup>8,105,106</sup>.

The effect of ART on the progression/regression of cervical lesions has until recently been unclear as studies have shown conflicting results. The currently best way to determine if ART is effective or not is to measure HIV-RNA levels. Unfortunately, most studies performed in this field have not had access to HIV-RNA levels and have therefore had to rely on self-reported ART use as a measure of effective ART, which may have led to reporting bias. Additionally, several studies have not adjusted for CD4 nadir, current CD4 count, or time on ART, which may explain why the association between ART and progression/regression of cervical lesion has remained uncertain for so long<sup>107,108</sup>. There are studies, however,

adjusting for CD4 count and time on ART that have shown an association between ART and outcome of CIN and recently a meta-analysis confirmed reduced incidence of HSIL/CIN2+, decreased progression of SIL, increased SIL/CIN regression and reduced incidence of ICC among women on ART<sup>78,103,109-111</sup>.

The dramatic decline seen for other ADCs (as mentioned above) when ART became available was initially not seen for ICC<sup>34</sup>. This is mainly believed to be due to increased life expectancy, essentially suggesting that before ART was available WLWH died of other AIDS-associated diseases that progress quicker than ICC. Registry studies in high-income countries now suggest a reduction of ICC in WLWH but in regions such as Eastern and Central Africa the incidence is increasing<sup>13,32,98</sup>.

### **3.4 TREATMENT OF ICC/CIN IN WOMEN LIVING WITH HIV**

Women with cervical cancer and its precursor CIN are recommended the same treatment irrespective of HIV status<sup>112</sup>. CIN1 is usually a self-healing lesion and is generally treated with expectancy only. CIN2 and CIN3 are most often treated with conization. Conization is a surgical procedure, which can be performed with local anaesthesia, where a cone-shaped wedge that includes the transformation zone and part of the endocervical canal is excised from the cervix uteri. Conization may be performed with an electrosurgical loop (LEEP; loop electrosurgical excision procedure or LLETZ; large loop excision of the transformation zone), which is presently the most common procedure, with cold knife conization or with laser.

Persistence and recurrence of CIN2/3 after treatment with conization is more common in WLWH and increases with the level of immunosuppression<sup>104,110,113,114</sup>. It is unclear if WLWH on ART have better outcome after conization than those not on ART as data is limited. A majority of studies previously performed have been based on self-reported use of ART as a measure of effective ART<sup>104,110,115</sup>.

Early stages of ICC are treated with hysterectomy, which is the surgical removal of the uterus. Locally advanced disease is most often treated with radio chemotherapy and for metastatic disease the treatment is usually palliative<sup>112,116</sup>. Mortality and relapse after treatment of ICC seems to be increased in WLWH, at least in LMIC but clinical data on WLWH and outcome after cervical cancer diagnosis is still scarce<sup>117-119</sup>.

### **3.5 CERVICAL CANCER SCREENING**

Cervical cancer screening has earlier been based on regular cervical cytology testing where the exfoliated cells from different CIN stages can be detected through microscopic examination. The detection and subsequent treatment of CIN abnormalities form the basis of the cervical cancer screening programs that have substantially decreased the incidence of ICC in high-income countries<sup>60,120</sup>. Unfortunately, cervical cancer screening coverage is still poor in many LMIC<sup>121</sup>. HPV-PCR-triaging has been gradually introduced in screening programs worldwide, though mainly in high-income-countries and in Sweden since 2017. For many countries, including Sweden, it is now recommended that women between the ages of 23 and 29 are tested with cervical cytology every three years, that women between 30 and 49 are tested for HR HPV every three years (with a complementing cervical cytology at the age of

41) and that women between 50 and 64 are tested for HR HPV every seven years<sup>122</sup>. For this thesis data is based on women who were recommended (if HIV-negative) cervical cytology testing every three (aged 23-50) to five years (aged 51-60)<sup>112</sup>.

### 3.5.1 CERVICAL CANCER SCREENING IN WOMEN LIVING WITH HIV

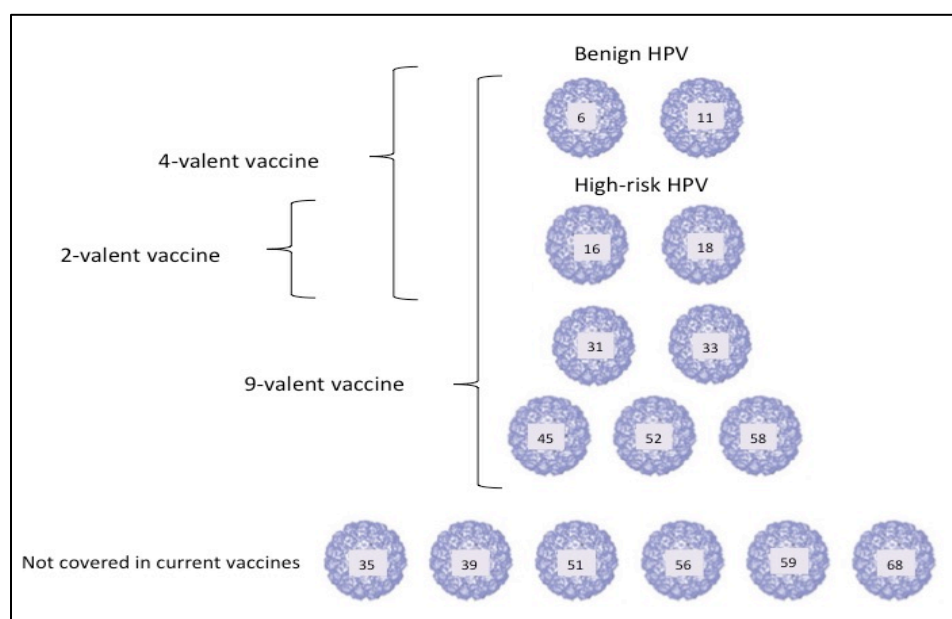
WLWH who have been adherent to regular cervical cancer screening (every six months in a US-based study and annually in a Danish-based study) do not have an increased incidence of ICC compared to HNW in studies with long-term follow-up<sup>123,124</sup>.

Until recently most high-income countries recommended WLWH to be screened with cervical cytology twice during the first year after HIV-diagnosis, followed by annual testing until the age of 60. This has also been the recommendation in Sweden for the time period included in this thesis. HPV-based screening in WLWH has not been recommended due to the high HPV prevalence in WLWH.

With increasing knowledge concerning HPV and HIV co-infection more guidelines are now leaning towards having the same/or more similar HPV-based screening programs for women independent of HIV-status<sup>65,112,125-127</sup>. This may reduce both under screening and over screening in WLWH<sup>128</sup>. While the consequences of under screening are clear, over screening is associated with an increased risk of pre-term birth (due to repeated conization) and increased anxiety in affected women<sup>129</sup>.

### 3.6 HPV VACCINATION

The HPV vaccine is based on the fact that the structural HPV protein (L1) is able to self-assemble into immunogenic virus-like particles. The vaccine induces neutralizing antibodies that prevent primary infection of the basal layer by binding and presenting HPV antigens to the cellular immune system thereby neutralizing the virus and creating a cellular memory<sup>130</sup>.



**Figure 7. HPV genotypes included in current HPV vaccines and high-risk HPVs not covered in current HPV vaccines.**

Initially there were two HPV vaccines, the 2-valent (bivalent) covering HPV 16 and 18 and the 4-valent (quadrivalent) vaccine covering HPV 6, 11, 16 and 18 (Figure 7). Later a 9-valent (nonavalent) vaccine, covering HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58, was launched. This is currently being used in HPV vaccination programs in some countries, such as the US and New Zealand<sup>130,131</sup>. All three vaccines have been proven safe and efficacious against 6-month persistent cervical infections of HPV 16 and HPV 18 and associated precancerous lesions and are equally recommended by the World Health Organization (WHO)<sup>132-134</sup>. The 9-valent may be cost-effective depending on the price of the vaccine and the setting<sup>131</sup>. Cross-protection against types 31, 33 and 45 has been seen for the 2-valent vaccine, more limited against type 31 for the 4-valent vaccine and it seems that high vaccination coverage is needed for cross-protection to appear<sup>133,135</sup>.

To date, 82 countries have introduced HPV vaccination of girls (9-14 years of age) in the national immunization programs with a mean vaccine coverage of 61% in those 52 countries with available data<sup>136</sup>. In Sweden, girls between the ages of 9 and 13 have been offered the 4-valent vaccine since 2012, with a current vaccine coverage of around 80%. In September 2018, it was decided that the 4-valent will now be exchanged for the 9-valent vaccine in Sweden. Vaccination of boys is not yet included in most national vaccination programs and whether this addition is cost-effective is still debated, especially where coverage of girls is above 80% as herd immunity is then assumed to cover boys too<sup>131</sup>. Adding boys to the vaccination program has been estimated cost-effective in the Swedish program but has not yet been implemented<sup>137</sup>.

### **3.6.1 HPV VACCINATION IN PEOPLE LIVING WITH HIV**

Both 2-valent and 4-valent HPV-vaccines have been found to be safe and well tolerated in the population living with HIV and do not alter the CD4 counts or HIV viral loads of those vaccinated<sup>138-141</sup>. The rate of seroconversion is similar in PLWH on suppressive ART and HIV negative individuals<sup>138,142,143</sup>. Low CD4 counts seem to be associated with a lower antibody titer response but data is limited and the clinical implication of this is unknown, as the protective titers of HPV antibodies are not defined<sup>142,144</sup>. Data regarding HPV vaccine efficacy in the HIV-infected population is scarce<sup>145</sup>.



## 4 AIM

### *General aim*

To study high-grade cervical intraepithelial neoplasia in women living with HIV in Sweden compared with HIV-negative women of the same region of birth.

### *Specific aims*

#### **Paper I**

- To assess the cumulative incidence, incidence rate and risk of CIN3+ in women living in Sweden, depending on HIV-status, frequency-matched for age and region of birth.

#### **Paper II**

- To analyse whether the prevalence of undiagnosed HIV in women diagnosed with CIN2+ in Sweden reaches the level suggested cost-effective for HIV-testing.
- To determine if the prevalence of undiagnosed HIV in migrant women diagnosed with CIN2+ in Sweden reaches the level suggested cost-effective for HIV-testing.

#### **Paper III**

- To study the outcome after treatment of CIN2+, depending on HIV-status and controlled for country of birth.
- To assess the predictors of treatment failure and recurrence, after treatment of CIN2+, in women living with HIV.

#### **Paper IV**

- To identify HPV-genotypes associated with CIN2+ in women living in Sweden, depending on HIV-status and controlled for country of birth.
- To determine the proportion of cases of CIN2+ that current HPV vaccines might have prevented, depending on HIV status and controlled for country of birth.

## METHODS

**Table 1. Summary of methods of included papers.**

	Study design	Final study population	Data collection	Main outcome	Data based on:
<b>Paper I</b>	Cohort	WLWH: 893 HNW: 205 842	1993-2011	Absolute risk of CIN3+ (cumulative incidence and incidence rate)  Relative risk of CIN3+ in WLWH compared to HNW (hazard ratio)	Registry linking
<b>Paper II</b>	Case-control	Case (CIN2+): 62 874 Control (no CIN2+): 897 703	1990-2014	Proportion of undiagnosed HIV in women with/without CIN2+	Registry linking
<b>Paper III</b>	Matched cohort	WLWH: 140 HNW 284	1983-2015	Treatment failure after treatment of CIN2+ (odds ratio)  Recurrence after treatment of CIN2+ (hazard ratio)	Registry linking  Medical record review
<b>Paper IV</b>	Case only	interim analysis	1983-2014	HPV-genotype detected in CIN2+ biopsies (prevalence ratio)	Registry linking  HPV-genotyping

Abbreviations: CIN3+: cervical intraepithelial neoplasia grade III, adenocarcinoma in situ and cervical cancer, CIN2+: cervical intraepithelial neoplasia grade II, grade III, adenocarcinoma in situ and cervical cancer, HNW: HIV-negative women; WLWH: women living with HIV

### 5.1 DATA SOURCES AND STUDY POPULATION

#### Registries used in this thesis

##### *The Swedish National HIV Registry (InfCare HIV)*

This registry includes > 99% of living Swedish residents diagnosed with HIV. Patients are consecutively enrolled to the registry at time of HIV diagnosis and demographic, therapeutic, and laboratory data are registered at least every six months<sup>38,40</sup>. InfCare HIV was established in 2003 and the implementation was finalized in all HIV care centres by 2008. Data regarding patients diagnosed before 2003 has been complemented retrospectively. Data quality and coverage is assessed regularly. For this thesis we extracted data regarding ART, HIV-RNA levels, CD4 counts, date of HIV-diagnosis, mode of HIV-transmission and country of birth.

##### *The Swedish National Cervical Screening Registry (NKCx)*

The NKCx includes all cervical cytology and histology reports in Sweden<sup>146</sup>. The completeness of the register is high,  $\geq 90\%$ , for both cytologies and histologies. Evaluation of data delivery and coverage is carried out annually. For some regions, screening data has been included since the 1960ies and the registry has had national coverage since 1993. For the counties of Stockholm and Gothenburg, from which the study populations in this thesis are based, data is complete since before 1990. For the women included in this thesis, all cervical cytology and histopathology reports of were collected.



## *The Swedish Population Registry*

The Swedish Population Registry (SPR) is maintained by the Swedish Tax Agency and it has records of all individuals residing in Sweden on a permanent basis. This registry includes data on births, immigration and emigration, deaths, marriages, divorces and changes of citizenship. At the time of birth or immigration the individual is assigned a personal identification number (PIN). For this thesis data was collected regarding date of birth, date of death, date of immigration or emigration and country of birth<sup>147</sup>.

### *Registry linkage procedure*

For all studies in this thesis we used the unique PIN assigned to all individuals in Sweden at birth or on immigration to link some (**Paper II**) or all (**Papers I, III and IV**) of above mentioned registries.

### **Study population**

In **Paper I** the study population consisted of all WLWH (n = 1284) who were alive and resident in the County of Stockholm at some point between 1993 and 2011 and HNW (n= 262 580) living in the same county during the same time period, frequency matched on age and region of birth. After inclusion criteria had been met (see paper I for details) the final study population included 893 WLWH and 205 842 HNW, between the ages of 18 and 60 years, with no prior history of cervical cancer and at least one registered cervical cytology or biopsy in the NKCx.

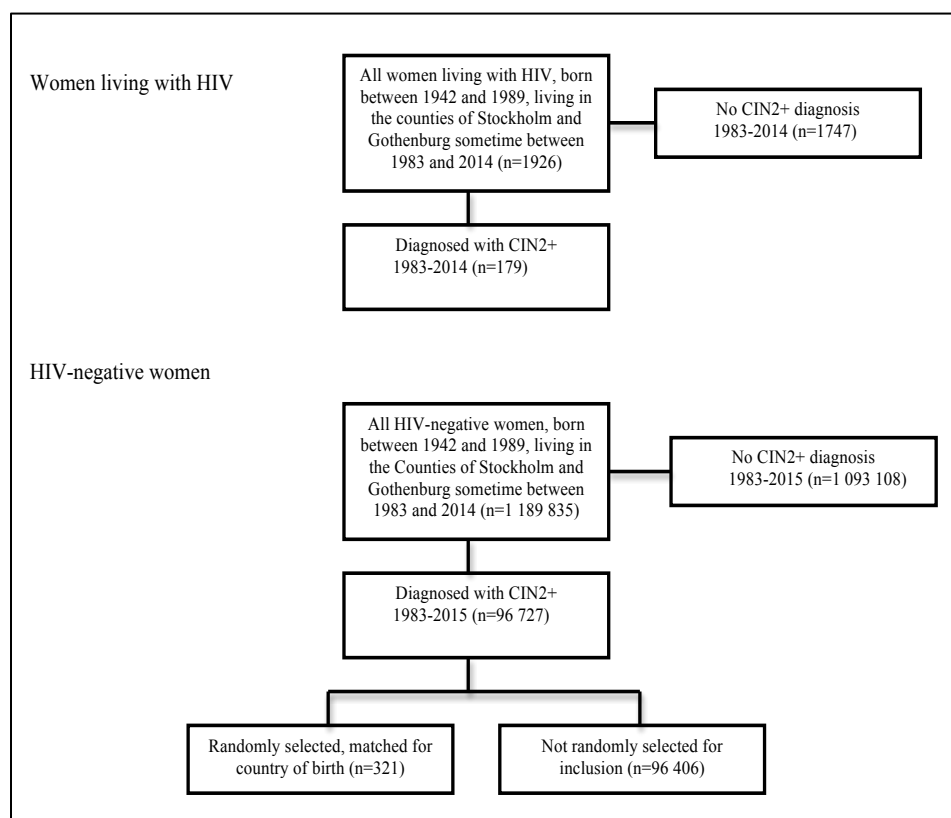
In **Paper II** the study population consisted of all women born between 1940 and 1990, residing in the counties of Stockholm or Gothenburg at some point between 1990 and 2014, with at least one cervical cytology or histology registered in the Swedish National Cervical Screening Register (NKCx) (n = 960 577) (Table 2).

**Table 2. Paper II. Study population.**

	No CIN2 <sup>a</sup>	Ever CIN2 <sup>b</sup>
All	897 703	62 874
<b>Region of birth<sup>c</sup></b>		
Sweden	641 467 (71.5)	52 834 (84.0)
Sub-Saharan Africa	19 808 (2.2)	495 (0.8)
Eastern Europe & Central Asia	61 220 (6.8)	2865 (4.6)
Asia & Pacific	33 192 (3.7)	1402 (2.2)
Latin America & Caribbean	19 674 (2.2)	925 (1.5)
Other/unknown	122 342 (13.6)	4353 (6.9)

a) At least one normal/mildly abnormal (cervical intraepithelial grade 1/atypical squamous cells of undetermined significance/atypical glandular cells/atypical cells of uncertain origin) cervical cytology/histology registered in the NKCx. b) At least one diagnosis of CIN2<sup>+</sup> (cervical intraepithelial neoplasia grade 2, grade 3 adenocarcinoma in situ and invasive cervical cancer) registered in the NKCx. c) According to UNAIDS definitions.

In **Papers III and IV** the study population consisted of all WLWH born between 1942 and 1989, living in the counties of Stockholm and Gothenburg sometime between 1983 and 2014 and diagnosed with CIN2+ (n = 179) and HNW as controls. For each WLWH, two HNW, living in the same counties sometime between 1983 and 2014, diagnosed with CIN2+ (n = 96 727), were randomly selected and matched for country of birth (n = 321) (Figure 8). For some WLWH, only one or no HNW from the same country of birth could be identified and a HNW from a neighbouring country was chosen instead. Some HNW matched to more than one WLWH.



**Figure 8. Flow chart of study population in paper III and IV.**

The final study population for **Paper III** consisted of 140 WLWH and 284 HNW, born between 1942 and 1989, randomly matched for country of birth, living in the counties of Stockholm and Gothenburg sometime between 1983 and 2014, treated for histology-verified CIN2+, with at least one follow-up cervical cytology/histology within one year and no hysterectomy performed before start of follow-up.

The final study population for **Paper IV** was not finalized at the time of printing this thesis. The interim analysis included 68 WLWH and 127 HNW, born between 1942 and 1989, randomly matched for country of birth, living in the counties of Stockholm and Gothenburg sometime between 1983 and 2014, with histology-verified CIN2+ and valid HPV-PCR result.

## Medical record review

For **Paper I** we reviewed 100 randomly selected medical records of included WLWH to assess whether there was an underreporting of cervical cytology in the NKCx for these

women. A medical record review was also performed for all women included in **Paper III** to collect information that was not registered in the registries. Variables collected included mode of treatment of CIN2+, radicality of conization performed, nativity and history of smoking.

## 5.2 ANALYSIS

### Regions of birth

For **Paper I** included women were classified into four regions of birth; *Sweden*, *Sub-Saharan Africa, East* (Eastern Europe and Central Asia and Asia and Pacific) and *Other*, using UNAIDS classification<sup>148</sup>.

For **Paper II** included women were classified into six regions of birth: *Sweden*, *Sub-Saharan Africa*, *Eastern Europe and Central Asia*, *Asia and Pacific*, *Latin America and Caribbean* and *Other* (Western Europe except Sweden, Canada, USA, North Africa, and Middle East). Women with an unknown country of birth were analysed together with women of birth region Other, as Other/unknown.

For **Paper III and IV** included women were classified into six regions of birth: *Sweden*, *Western Europe except Sweden*, *Eastern Europe and Central Asia*, *Sub-Saharan Africa*, *Asia and Pacific*, and *Latin America and Caribbean*.

### The CD4+ trajectory model

For **Paper II** we applied a CD4+ trajectory model to estimate the time of HIV acquisition. The model extrapolates the CD4-cell decline backwards from the CD4 count at diagnosis to estimate a probable date of HIV-transmission. This method was first developed by Public Health England in collaboration with the European Centre for Disease Prevention and Control (ECDC) in 2012 and has later been revised and improved<sup>149,150</sup>. The model is based on a large group of British HIV-seroconverts (where time of seroconversion was known because of frequent HIV-testing among people at risk), whereby an algorithm was designed to estimate the time of HIV-seroconversion, based on factors associated with the intercept and the slope of CD4-cell decline. Region of birth and age, both known to affect CD4-cell decline, are adjusted for in this model.

After applying the CD4+ trajectory model, the time of estimated HIV-seroconversion for each included woman was divided into three estimates: the earliest probable time of seroconversion, the average probable time, and the latest probable time.

## HPV-genotyping analysis

### *Collection of archived cervical material and sectioning*

For **Paper IV** all archived diagnostic slides from cervical biopsies/conizations of included women were retrieved and reviewed by a senior pathologist to reconfirm histological diagnosis. Formalin-Fixed-Paraffin-Embedded (FFPE) blocks from cervical biopsies/conizations of confirmed cases of CIN2+ were then collected from the bio banks where samples are stored in local pathology laboratories. All cases were subsequently sectioned at accredited laboratories, according to a contamination-proof procedure<sup>151</sup>. The first and last sections for each case were stained with haematoxylin and eosin (H&E-staining) for later re-review if needed. In between each case-block, a blank-block was sectioned as contamination control.

### *DNA extraction and genotyping*

All sections, blank-blocks and case blocks, were extracted with a xylene-free method and HPV genotyped using polymerase chain reaction (PCR) with modified general primers (MGP)-PCR (primer targeting L1) and hybridization with type-specific probes in Luminex<sup>151,152</sup>. Forty-two beads, encompassing 37 different HPV-types, 3 HPV variants, and two “universal” HPV probes, were included in the Luminex. Luminex is a magnetic bead-based multiplex immunoassay. The beads are fluorescently labelled, each with a distinct colour code to permit discrimination between different HPV-genotypes (Figure 9).



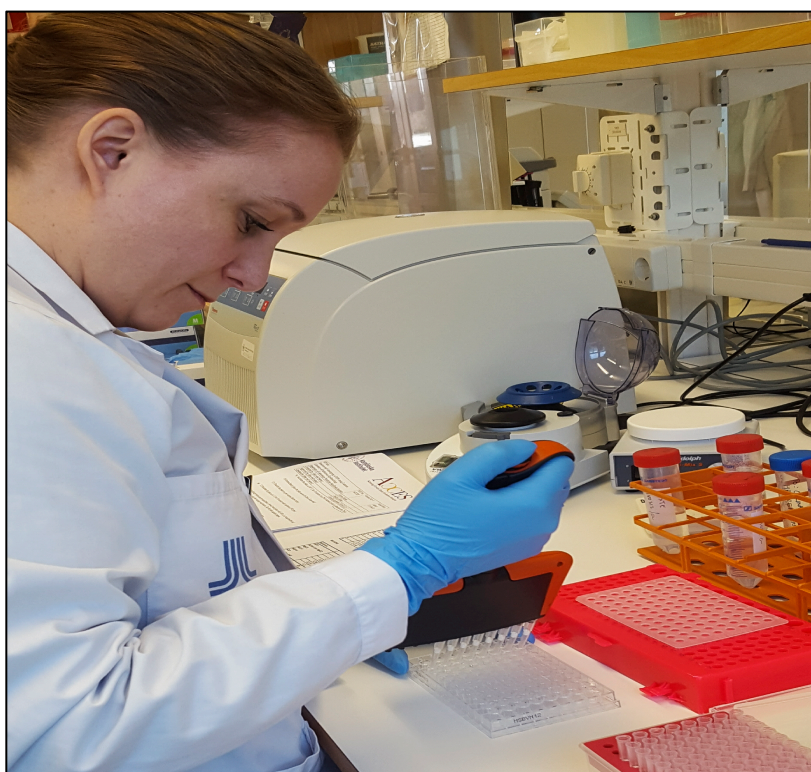
**Figure 9.** HPV-type specific probes coupled to coloured beads.

If the case was HPV-negative, extracted material from both the blank-block and case-block was diluted 1/10 and re-tested. Blank-blocks and the matched case-blocks were treated in exactly the same way during the whole process. All samples were analysed for beta-globin by quantitative real-time PCR to confirm sample adequacy<sup>153</sup>. The blank-block had to be negative for both HPV and beta-globin and the case-block positive for beta-globin. Cases that were beta-globin negative were classified as inadequate samples and were not analysed and neither were cases coupled to contaminated blank blocks (Figure 10).

### *Quantitative real-time PCR for HPV 16 and HPV 18*

Cases that were HPV negative in genotyping were analysed for the E6/E7 regions of the two common oncogenic HPV types, HPV 16 (primer targeting E7) and HPV 18 (primer targeting E6), using real-time PCR with one  $\mu\text{L}$  DNA used in both essays with a total volume of 25  $\mu\text{L}$ <sup>63</sup>.

The laboratory was blinded to the identity of the samples and whether samples were from WLWH or HNW.



**Figure 10. Camilla (co-author paper IV) preparing for HPV-genotyping.**

### **Definition of outcomes**

For **Paper I** the main outcome was **CIN3+**, defined as CIN3, adenocarcinoma in situ or invasive cervical cancer. In a separate analysis main outcome was defined as CIN2+. Inclusion-date for WLWH was defined as the 1st of January 1993, date of HIV diagnosis or when the subject turned 18 years, whichever came last. Inclusion-date for HNW was defined as the 1st of January 1993 or when the subject turned 18 years, whichever came last. All subjects were followed from inclusion date until diagnosis of CIN3+ (or in a separate analysis CIN2+), last registered cervical cytology/biopsy or December 31, 2011, whichever happened first. Only histological results were used for assessing the outcome.

For **Paper II** the main outcome was **proportion of undiagnosed HIV** among women diagnosed with or without CIN2+. After linking the study population to InfCare HIV, all women with CIN2+ diagnosed before HIV diagnosis and all women with a normal or mildly abnormal (defined as any of: CIN1, atypical squamous cells of undetermined significance,



atypical glandular cells, atypical cells of uncertain origin) cervical cytology or histology before HIV diagnosis were identified. After applying the CD4+ trajectory model, the time of estimated HIV acquisition for each included woman was divided in three estimates: the earliest probable time of acquisition, the average probable time, and the latest probable time. A woman was defined as having an undiagnosed HIV at the time of CIN2+ diagnosis if CIN2+ was diagnosed sometime between the year of the earliest probable date of HIV acquisition and the date of HIV diagnosis. Women diagnosed with CIN2+ before the year of the earliest probable date of HIV acquisition were excluded.

For **Paper III** the main outcome was **treatment failure** defined as presence of CIN2+ on cervical cytology/histology at initial follow-up, within one year after treatment of CIN2+. Women with normal cervical cytology/histology at follow-up were considered successfully treated and were included in analysis of **recurrence**, defined as subsequent CIN1+. These women were followed from date of first follow-up after treatment of CIN2+, until date of recurrence, or if no recurrence took place, until date of last registered cervical cytology/histology.

For **Paper IV** the main outcome was **HPV genotype** detected in women diagnosed with CIN2+ depending on HIV status.

## Statistical analysis

**Table 3. Summary of statistical analysis of included papers.**

	Study design	Measures	Statistical Methods
<b>Paper I</b>	<ul style="list-style-type: none"> <li>• <b>Cohort.</b></li> <li>• HNW were matched to WLWH by age and region of birth.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Cumulative incidence</b> of CIN3+.</li> <li>• <b>Incidence rate</b> of CIN3+.</li> <li>• <b>Hazard ratio</b> (with 95% CI) of CIN3+ in WLWH vs. HNW.</li> <li>• <b>Distribution</b> of CD4 nadir depending on outcome.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Cox regression.</b> Stratified for age at inclusion, calendar year at inclusion and region of birth. Adjusted for number of cervical cytology tests. WLWH alone adjusted for CD4 count at inclusion and nadir CD4.</li> <li>• <b>Likelihood ratio test.</b> Interaction test for HIV status and birth region.</li> <li>• <b>Kernel average smoother</b> (nested sub analysis).</li> </ul>
<b>Paper II</b>	<ul style="list-style-type: none"> <li>• <b>Case-control.</b></li> <li>• Un-matched.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Proportion and relative risk</b> (with 95% CI) of undiagnosed HIV in women with/without CIN2+.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Chi-square</b> or <b>Fisher</b> test.</li> <li>• <b>Wilcoxon rank sum</b> test.</li> </ul>
<b>Paper III</b>	<ul style="list-style-type: none"> <li>• <b>Matched cohort.</b></li> <li>• HNW were matched to WLWH by country of birth.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Odds ratio</b> (with 95% CI) for treatment failure in WLWH vs. HNW.</li> <li>• <b>Hazard ratio</b> (with 95% CI) for recurrence in WLWH vs. HNW.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Logistic regression</b> to estimate effect of covariates on treatment failure. All models adjusted for age (continuous, at time of treatment of CIN2+) and region of birth. WLWH alone adjusted for HIV-RNA level, CD4 count at inclusion and CD4 nadir.</li> <li>• <b>Cox regression</b> to estimate effect of covariates on recurrence. Same adjustments as above.</li> </ul>
<b>Paper IV</b>	<ul style="list-style-type: none"> <li>• <b>Case only.</b></li> <li>• HNW were matched to WLWH by country of birth.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Prevalence ratio.</b> Comparison of type-specific HPV-genotype prevalence in WLWH vs. HNW.</li> </ul>	<ul style="list-style-type: none"> <li>• <b>Poisson regression.</b> All models adjusted for age (continuous, at time of CIN2+ diagnosis), grade of cervical lesion, and region of birth.</li> </ul>

Abbreviations: CI: confidence interval, CIN3+: cervical intraepithelial neoplasia grade III, adenocarcinoma in situ and cervical cancer, CIN2+: cervical intraepithelial neoplasia grade II, grade III, adenocarcinoma in situ and cervical cancer, HIV+: living with HIV, HNW: HIV-negative, nadir CD4: lowest CD4 count ever measured for that individual, WLWH: women living with HIV.

In **Paper I** we calculated the incidence rate, cumulative incidence of CIN3+ as estimates for the absolute risk of CIN3+ (and in a sensitivity analysis CIN2+) in the studied cohort. Prevalent cases were excluded (CIN2+ diagnosed within 6 months of inclusion). Cox regression models, stratified for age at inclusion, calendar year at inclusion and region of birth, were used to estimate relative risks through hazard ratios (with corresponding 95% confidence intervals) of CIN3+ (and in a sensitivity analysis CIN2+) in WLWH compared to HNW. Adjustment was made for number of cervical cytology tests taken. Differences in hazard ratios between birth regions were tested for interaction (likelihood ratio test) between HIV status and birth region.

In a sub analysis including only WLWH, hazard ratios of CIN3+ (and separately CIN2+) were estimated and adjusted for CD4 count at inclusion and nadir CD4. To illustrate the distribution of nadir CD4 in WLWH with or without CIN2+ (and separately CIN3+) a nested sub analysis was performed using a Kernel average smoother matched one-to one for age, birth region and time of follow-up.

In **Paper II** the time of HIV acquisition was estimated using a CD4+ trajectory model (described above). We calculated the proportion and relative risk, with corresponding 95% confidence intervals (CI), of undiagnosed HIV in women with a diagnosis of CIN2+ compared to women with no diagnosis of CIN2+. Tests for significant differences in proportions were performed using the Chi-square test or, when numbers were small, the Fisher test. Differences in time to HIV diagnosis and level of nadir CD4 were tested for significance using Wilcoxon rank sum test. All other continuous variables were normally distributed and differences were calculated using the t-test.

In **Paper III** logistic regression was used to estimate the effect of covariates (HIV-status, grade of cervical lesion, treatment modality, nativity, positive surgical margins and smoking) associated with treatment failure in all women and in WLWH only (suppressive ART and CD4 count at time of CIN2+ treatment, nadir CD4, mode of HIV-transmission and decade of HIV-diagnosis) through odds ratios (with corresponding 95% CI). Being highly dependent covariates, CD4 count and HIV-RNA levels (at time of treatment of CIN2+) were not adjusted for in the same model. All models were adjusted for age at time of treatment of CIN2+ and region of birth.

Cox regression was used to estimate the effect of covariates on recurrence (with the same covariates as for treatment failure) through hazard ratios (with corresponding 95% CI). Schoenfeldt residual plots were used to ensure that the proportional hazards assumption was not violated. Suppressive ART was defined as HIV-RNA <50 copies/mL at time of treatment of CIN2+ (and in a sensitivity analysis <500 copies/mL).

In a sub analysis only WLWH with suppressive ART were compared to HNW. In another sub analysis WLWH with more than 6 months suppressive ART at time of treatment of CIN2+ were compared to those with less than 6 months suppressive ART.

In **Paper IV** type-specific HPV prevalence of specific HR HPV types detected in CIN2+ was compared between women with or without HIV. Prevalence ratios (PR) were calculated using generalized linear models (Poisson regression) with 95% CI. All models were adjusted for age (continuous, at time of CIN2+ diagnosis), grade of cervical lesion, and region of birth.

In order to estimate the proportion of cases of CIN2+ that might have been prevented by current HPV vaccines, the proportion of HR HPV types covered by the 2-valent/4-valent HPV vaccines (HPV 16 and/or 18) and the 9-valent HPV (16/18/31/33/45/52/58) vaccine was calculated.

Calculations were done using STATA 13 software.

### **Ethical considerations**

All four studies were approved by the Regional Ethical Review Board in Stockholm, Sweden (Diary numbers 2012/70-31/1 + 2012/1176-32+2013/2032-32+2015/1970-32+2016/1618-32). The ethical board determined that, due to the population-based nature of the study, informed consent from study participants (including medical record review) was not required.



## 6 RESULTS

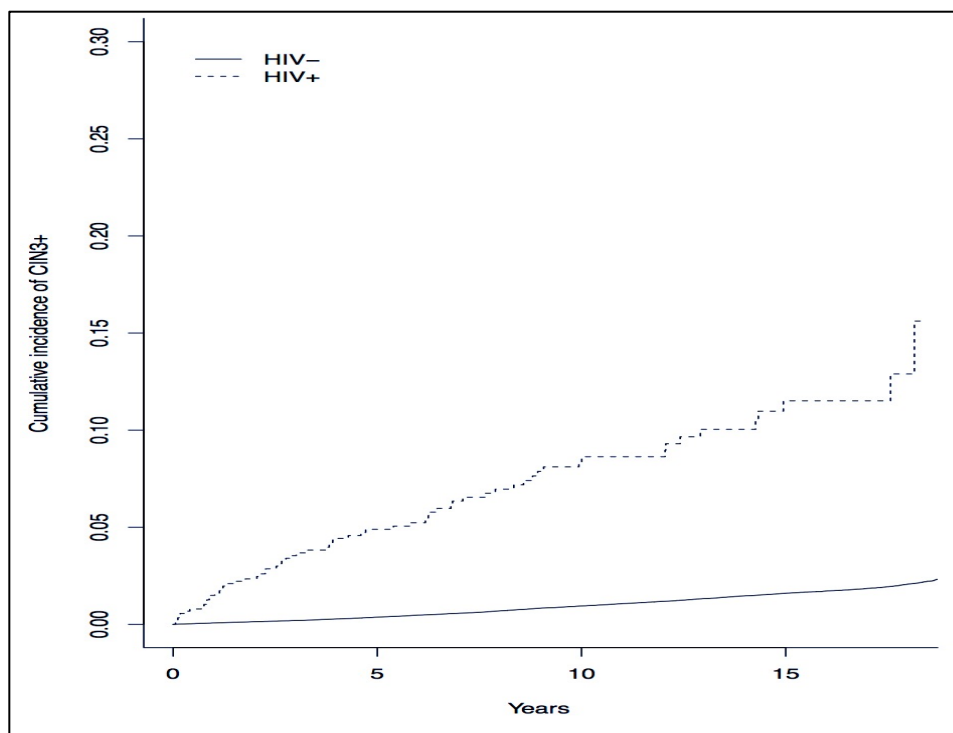
### Study population

WLWH included in the final study populations (although different for all papers) included in this thesis were all dominated by migrants, around 70%. Most WLWH had acquired HIV heterosexually (>80%) and a majority had at one point been highly immunosuppressed with a median nadir CD4 < 200 cells/ $\mu$ l.

### 6.1 PAPER I

A total of 893 WLWH and 205 842 HNW contributed with 7587 and 2 815 303 years respectively and during that time 65 WLWH and 3129 HNW were diagnosed with CIN3+.

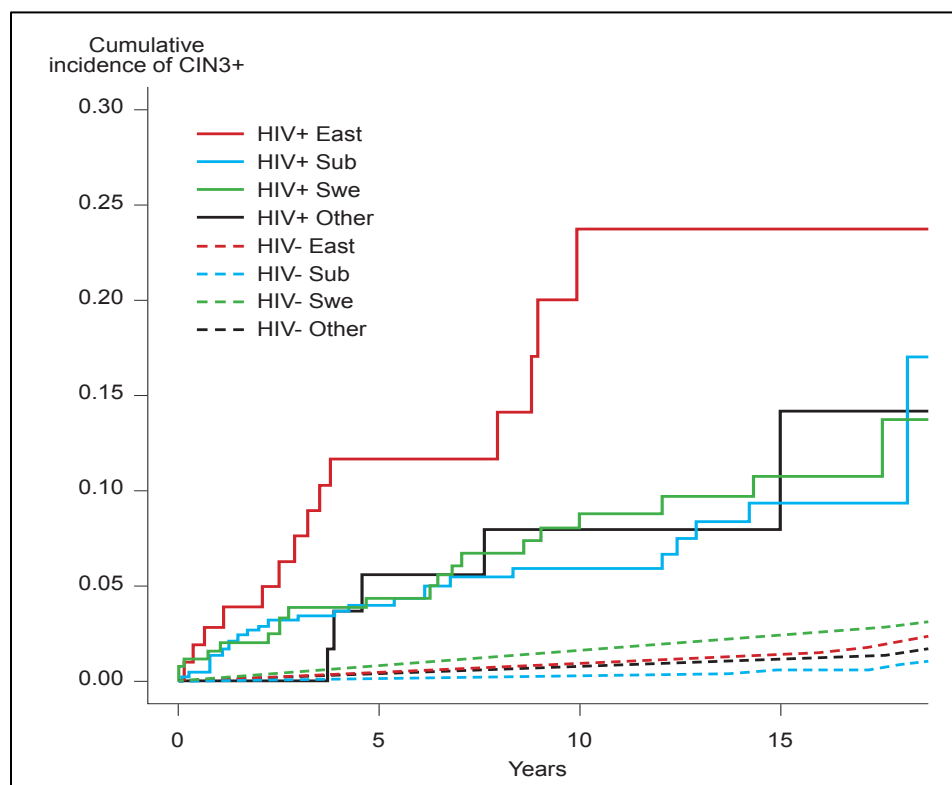
#### *Incidence of CIN3+*



**Figure 11. Paper I. Cumulative incidence of CIN3+ in women living with HIV (HIV+) and HIV-negative women (HIV-).**

We found the incidence of CIN3+ to be higher in WLWH than in HNW. After 18 years of follow-up the cumulative incidence (CuI) of CIN3+ was 13.1% (95% CI 8.9-17.2) in WLWH vs. 2.1% (95% CI 2.0-2.2) in HNW (Figure 11). The incidence rate was 8.6 (95% CI 6.7-10.9) vs. 1.1 (95% CI 1.1-1.2) per 1000 person years in WLWH and HNW, respectively.

The highest incidence of CIN3+ was seen in WLWH born in the region classified as East (Eastern Europe & Central Asia and Asia & Pacific), which was dominated by women born in Thailand (70%). After eight years of follow up these women had a CuI of 14.1% (95% CI 5.9-22.3) compared with a low CuI of 0.5% (95% CI 0.4-0.6) in HNW from the same region (Figure 12).



**Figure 12. Paper I. Cumulative incidence of CIN3+ in women living with HIV (HIV+) and HIV-negative (HIV-) women by region of birth (Swe, Sweden; Sub, Subsaharan Africa; East, Eastern Europe & Central Asia and Asia & Pacific; Other).**

#### *Risk of CIN3+*

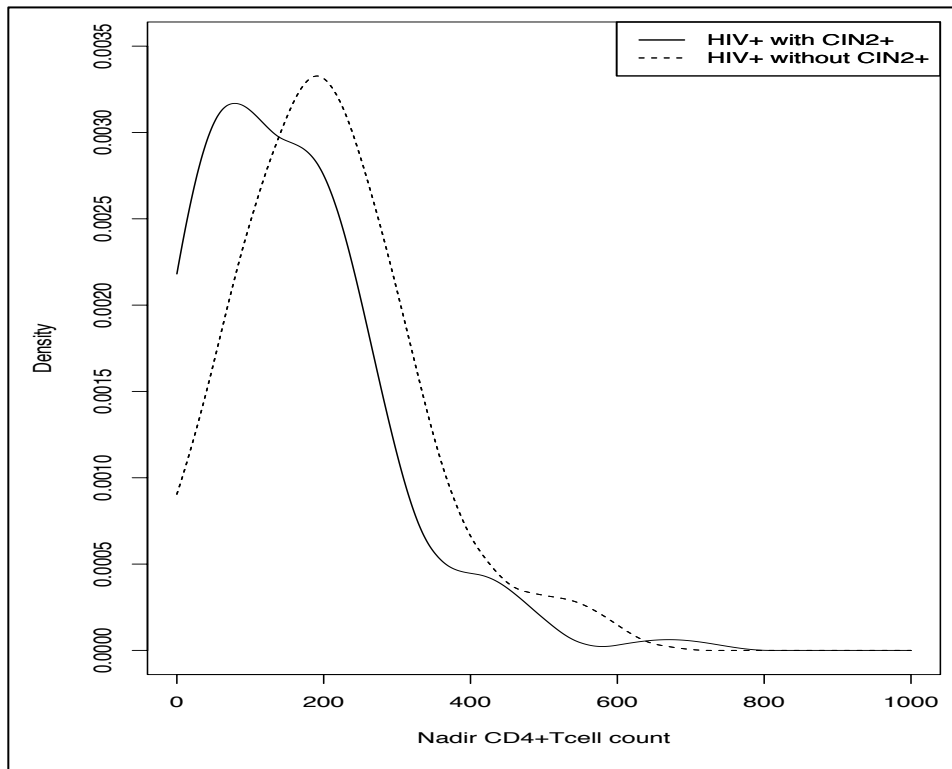
We found WLWH to have almost nine times higher risk of CIN3+ than HNW (HR 8.8, 95% CI 6.9-11.3). There was a significant interaction between being HIV-infected and birth region ( $p = 0.024$ ), with the least difference in risk seen between WLWH and HNW born in Sweden.

#### *Risk of CIN3+/CIN2+ in WLWH only*

A CD4 count at inclusion above 349 was shown to be protective against both CIN3+ ( $CD4^+$  200-349: HR 0.58: 95% CI 0.29-1.15;  $CD4^+$  350-500: HR 0.40: 95% CI 0.18-0.94;  $CD4^+$  > 500: HR 0.48: 95% CI 0.23-0.98 vs.  $CD4^+$  < 200: HR 1.0, ref) and CIN2+ (see paper I). Decreasing nadir CD4 count was significantly associated with CIN2+ only ( $p = 0.0001$ ), graphically illustrated in a nested sub analysis (Figure 13).

WLWH born in the East region (70% from Thailand) had more than two times higher risk of CIN3+ compared with WLWH born in Sweden (HR 2.5: 95% CI 1.22-5.02). When adjusting for nadir CD4 (HR 2.4: 95% CI 1.16-4.76) and CD4 at inclusion (HR 1.78: 95% CI 0.80-3.94) the increased risk remained, although non-significantly for the latter adjustment.

As the prevalence of current or prior ART at time of inclusion was very low (9%), adjustment for time on ART or time with suppressive ART was not performed in paper I.



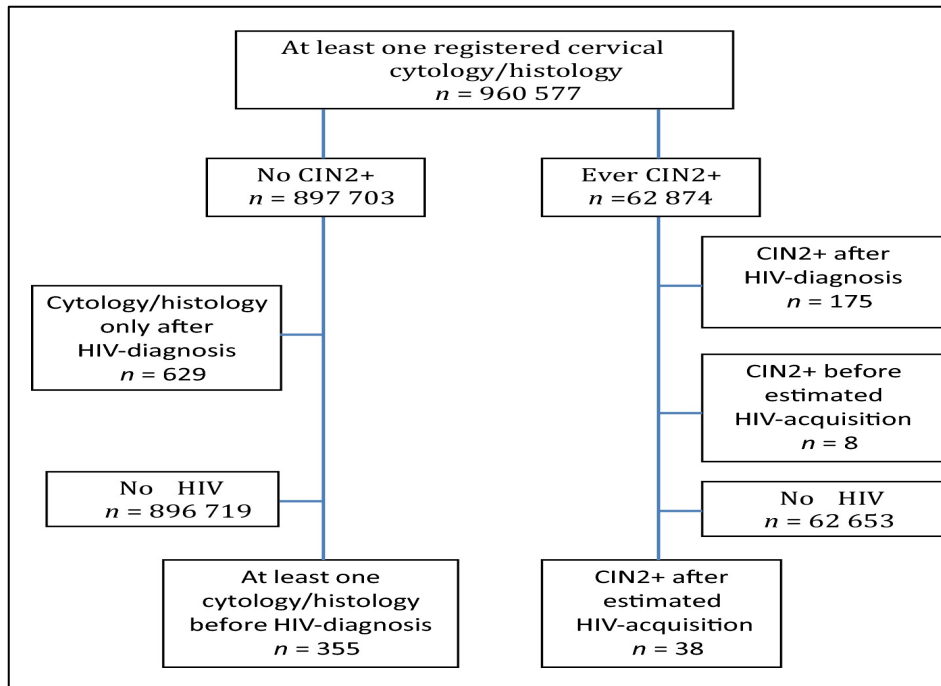
**Figure 13. Paper I. The distribution of nadir CD4+ T-cell count/ $\mu$ L in HIV-infected with an outcome of CIN2+ compared with HIV-infected with no outcome in a nested sub-analysis, matched for age (5-year interval), birth region and time of follow-up.** The area under the curve shows the fraction of cases with an outcome of CIN2+ (or no outcome) that had nadir CD4 counts within a given interval.

#### *Registered cytology tests*

WLWH had a median number of registered cytology tests of one (interquartile range [IQR] 1-4) during a median follow-up of seven years, while HNW had a median number of 4 tests (IQR 2-7) during a median follow-up of 15 years. Adjustment for number of registered tests made no difference to our results (data not shown).

## 6.2 PAPER II

A total of 960 577 women were included out of whom 62 874 had at least one registered diagnosis of CIN2+ and of these women 175 were diagnosed with CIN2+ after HIV diagnosis and 62 653 had no HIV diagnosis (Figure 14). After applying the CD4+ trajectory model, 38 women were defined as having had undiagnosed HIV at the time of CIN2+ diagnosis (Figure 14).



**Figure 14. Paper II. Flowchart of the study population.**

The proportion of undiagnosed HIV was higher in women with CIN2+ than in women without but did not reach the threshold of 0.1% (Table 4). Among migrants, the proportion of undiagnosed HIV was also higher among women with CIN2+ than among women without and exceeded 0.1% (Table 4).

Women with undiagnosed HIV at the time of CIN2+ had a significantly lower nadir CD4 count compared with women without CIN2+ (median nadir CD4 95 cells/ $\mu$ l vs. 210 cells/ $\mu$ l;  $P < 0.01$ ). Women born in the birth region Asia and Pacific, dominated by Thai women (80%), had a particularly low median nadir CD4 count when HIV was undiagnosed at the time of CIN2+ compared with women born in the same region without CIN2+ before HIV diagnosis (25 cells/ $\mu$ l vs. 180 cells/ $\mu$ l;  $P < 0.01$ ).

**Table 4. Paper II. Proportion [% (95% CI)] of undiagnosed HIV at the time of CIN2<sup>+</sup> compared with women with no CIN2<sup>+</sup>.**

	Undiagnosed HIV at time of CIN2+ % (95% CI)	Undiagnosed HIV and no CIN2+ % (95% CI)	P-value
All	0.06 (0.04-0.08)	0.04 (0.04-0.04)	0.017
Migrant	0.30 (0.20-0.43)	0.08 (0.07-0.10)	<0.001
Sweden	0.02 (0.01-0.04)	0.02 (0.02-0.02)	0.29
Sub-Saharan Africa	3.64 (2.24-5.80)	0.71 (0.60-0.84)	<0.001
Eastern Europe & Central Asia	N/A	0.01 (0-0.02)	N/A
Asia & Pacific	0.71 (0.36-1.35)	0.13 (0.10-0.18)	<0.001
Latin America & Caribbean	0.22 (0.04-0.87)	0.05 (0.03-0.10)	0.035
Other/unknown	N/A	0.01 (0.01-0.02)	N/A
Thailand	2.02 (0.94-4.10)	0.57 (0.41-0.78)	<0.001
Ethiopia	5.26 (2.16-11.57)	0.85 (0.60-1.19)	<0.001

### 6.3 PAPER III

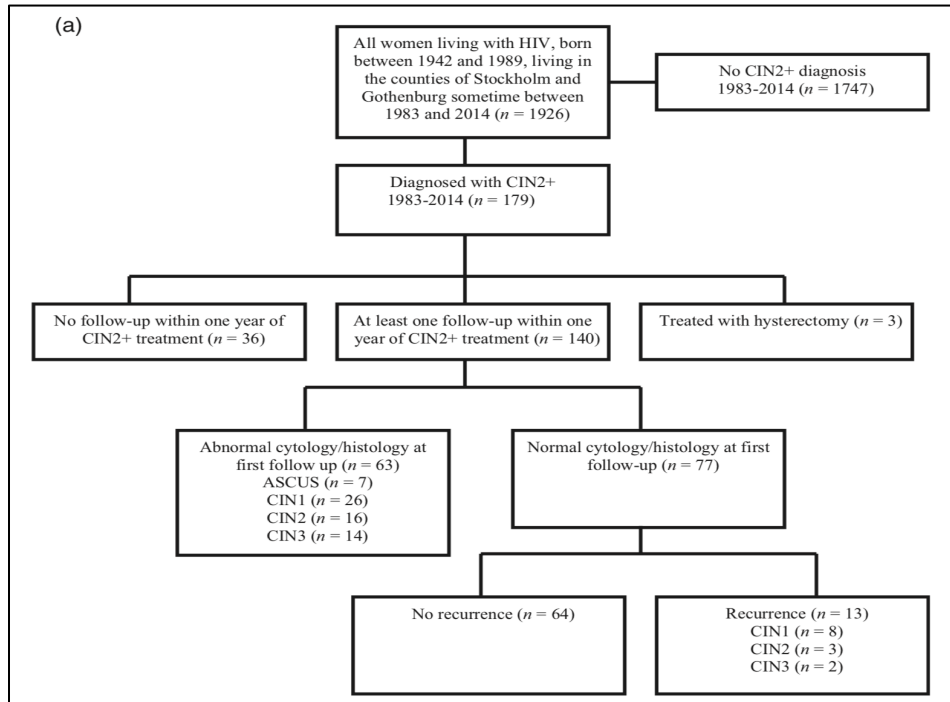


Figure 15. Paper III. (a) Flowchart of WLWH included in the study population.

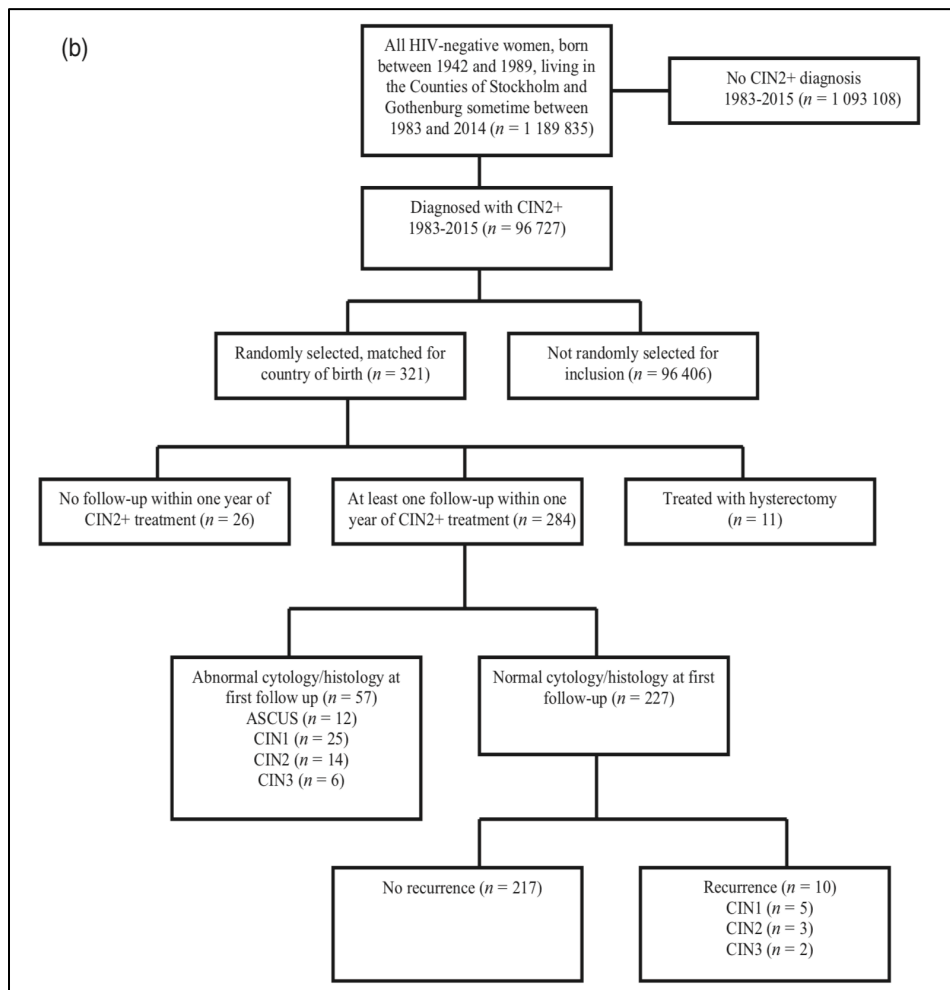


Figure 16. Paper III. (b) Flowchart of HIV-negative women included in the study population.

A total of 140 WLWH and 284 HNW were included in the final study population (Figure 15, Figure 16). Sixty-five percent of WLWH were on at least three antiretrovirals at time of treatment of CIN2+ and 53% on suppressive ART.

Among included women, 21% of WLWH and 7% of HNW had treatment failure defined as CIN2+ at first follow-up. Thus, WLWH were more than three times more likely to have treatment failure after treatment of CIN2+ than HNW (OR 3.7: 95% CI 2.0-6.8) (Table 5). Grade of lesion but not treatment modality or nativity was significantly associated with treatment failure in logistic regression analysis.

Suppressive ART (HIV-RNA < 50 copies/mL) was associated with reduced likelihood of treatment failure (OR 0.3: 95% CI 0.1-0.8) and this remained after adjusting for nadir CD4 count (Table 5).

**Table 5. Paper III. Characteristics associated with treatment failure.**

CIN2+ at follow-up	Odds ratio (95% CI)
WLWH vs. HNW	3.7 (2.0-6.8)
WLWH vs. HNW, adjusted for grade of initial lesion	3.9 (2.1-7.2)
WLWH with suppressive ART vs. HNW	1.8 (0.8-4.2)
Only WLWH	
Suppressive antiretroviral therapy	
Suppressive ART (defined as HIV-RNA<50) vs. HIV-RNA >50 copies/ml	0.3 (0.1-0.8)
Suppressive ART (defined as HIV-RNA<500) vs. HIV-RNA>500 copies/ml	0.3 (0.1-0.7)
Suppressive ART vs. HIV-RNA >50, adjusted for nadir CD4 count	0.3 (0.1-0.7)
Suppressive ART >6 months vs. <6 months	1.1 (0.3-5.3)
Suppressive ART >6 months vs. <6 months, adjusted for nadir CD4 count	1.1 (0.2-5.1)
CD4 count, cells/μL	
<200	8.5 (2.3-30.7)
200-349	0.9 (0.2-4.0)
350-499	1.3 (0.3-5.1)
≥500	1 (ref)
Nadir CD4 count, cells/μL	
<200	6.5 (0.8-53.6)
200-349	2.7 (0.3-26.0)
≥350	1 (ref)
Nadir CD4 count <200 compared to ≥350, adjusted for CD4 count (cont.)	2.9 (0.3-27.9)

Estimated through odds ratios in WLWH compared with HNW, controlled for age and region of birth.

Advanced immunosuppression (CD4 count < 200 cells/ $\mu$ l) at time of treatment of CIN2+ was associated with more than eight times higher odds ratio of treatment failure compared with CD4 count  $\geq$  500 (OR 8.5: 95% CI 2.3-30.7). Meanwhile, level of nadir CD4 count was not significantly associated with the likelihood of treatment failure. When adjusting for CD4 count at inclusion and nadir CD4 count in the same model the association between level of nadir CD4 count and treatment failure decreased even more (Table 5).

Of the 304 women included in the analysis of recurrence (defined as CIN1+), 13 out of 77 (17%) WLWH and 10 out of 227(4%) HNW where defined as having recurrence. WLWH were five times more likely to recur (hazard ratio 5.0, 95% CI 2.1-11.6). The only statistically significant variable associated with recurrence, in Cox regression analysis, was CD4 count (continuous) at time of treatment of CIN2+ ( $P_{\text{trend}}$ =0.0347).



## 6.4 PAPER IV

In this interim analysis 68 WLWH and 127 HNW had valid HPV genotype results out of which 64 (94%) WLWH and 116 (91%) HNW were HPV positive. Fifty-nine percent were on at least three antiretroviral drugs at the time of CIN2+ diagnosis and 47% on suppressive ART (defined as HIV-RNA <50 copies/mL).

### *Single HPV infections*

Although HPV 16 was the most common single HR HPV infection of both WLWH (22%) and HNW (43%), WLWH were less likely to be infected with HPV 16 than HNW (PR = 0.6, 95% CI: 0.35-0.97, Table 6). HPV 35 was the second most common single HR HPV infection in WLWH (11%) compared to HNW (3%) (PR = 4.0, 95% CI: 0.94-16.67). Most WLWH with HPV 35 were born in sub-Saharan Africa (5/7). Both HPV 18 and 45 were less common than expected in both WLWH and HNW (Table 6).

**Table 6. Comparison of HPV genotypes detected in HPV-positive women diagnosed with CIN2/CIN3/AIS/ICC stratified by HIV status.**

	WLWH n (%)	HNW n (%)	Prevalence ratio <sup>1</sup> (95% CI)
Any positive HPV	64	116	
HR single (group 1/2A <sup>2</sup> )			
HPV 16	14 (22)	50 (43)	0.6 (0.35-0.97)
HPV 35	7 (11)	3 (3)	4.0 (0.94-16.67)
HPV 18	2 (3)	3 (3)	1.0 (0.16-6.99)
HPV 45	2 (3)	3 (3)	1.2 (0.19-7.16)
HPV 33	2 (3)	7 (6)	0.6 (0.12-2.50)
HPV 52	2 (3)	7 (6)	0.5 (0.09-2.43)
HPV 31	2 (3)	7 (6)	0.5 (0.12-2.02)
HPV 58	2 (3)	1 (1)	3.8 (0.34-41.44)
HPV 51	2 (3)	1 (1)	3.0 (0.21-42.39)
HPV 39	-	-	-
HPV 56	-	-	-
HPV 59	-	-	-
HPV 68	-	-	-
Multiple HPV			
1/2A and/or 2B and/or benign	28 (44)	26 (22)	2.0 (1.30-3.10)
Multiple including ≥ 1 HR HPV	27 (42)	25 (22)	2.0 (1.27-3.13)
Any multiple HR HPV (HR+HR)	18 (28)	15 (13)	2.1 (1.17-3.79)
Multiple HR including HPV 16	6 (9)	9 (8)	1.2 (0.46-3.22)
Multiple HR including HPV 18	2 (3)	1 (1)	2.4 (0.35-16.58)
Multiple HR non HPV 16/18 (HR+HR)	10 (16)	5 (4)	3.7 (1.39-9.78)
Potential HPV vaccine coverage			
HPV 16/18 alone <sup>4</sup> (bivalent vaccine)	16 (25)	55 (47)	0.6 (0.38-0.97) crude 0.5 (0.33-0.84)
At least one of HPV 16/18	26 (41)	69 (59)	0.7 (0.53-1.02) crude 0.7 (0.49-0.95)
HPV 16/18/31/33/45/52/58 alone <sup>4</sup> (9-valent vaccine)	33 (52)	93 (80)	0.7 (0.53-0.88) crude 0.6 (0.50-0.83)
At least one of 16/18/31/33/45/52/58	47 (73)	103 (89)	0.9 (0.73-1.02) crude 0.8 (0.7-0.98)

Numbers are n (% of any positive HPV). Percentages do not always add up to a hundred due to rounding.

<sup>1</sup>Prevalence ratio (PR) (HIV-infected versus HIV-negative women) calculated using Poisson regression analysis, adjusted for age, grade of lesion and region of birth. <sup>2</sup> 1/2A including HPV 16/18/31/33/35/39/45/56/58/59/68. <sup>3</sup> 2B including 26, 30, 53, 66, 67, 69, 70, 73, 82. <sup>4</sup>Single or multiple infections with specified genotypes only.

### *Multiple HPV infections*

Multiple infections with at least one HR HPV genotype were significantly more common in WLWH (42%) than in HNW (22%) (PR = 2.0, 95% CI 1.27-3.13) and so were multiple infections with two or more HR HPV genotypes (PR = 2.1, 95% CI 1.17-3.79, Table 6). WLWH were more likely to have multiple HR HPV infections not including HPV 16 or 18 than HNW (PR = 3.7, 95% CI 1.39-9.78).

### *HPV types represented in current HPV vaccines*

Only 25% of WLWH vs. 47% of HNW had HR HPV types that are covered by the 2-valent and 4-valent HPV vaccines (HPV 16 and/or 18) (PR = 0.6, 95% CI: 0.38-0.97). Even HR HPV types covered for by the 9-valent HPV vaccine (16/18/31/33/45/52/58) were significantly less frequent in WLWH (52%) compared to HNW (80%) (PR=0.7, 95% CI 0.53-0.88, Table 6). When assuming that lesions with multiple infections, including HPV 16/18 are caused by the latter there was no longer a significant difference between WLWH compared to HNW (Table 6).

A more severe grade of cervical lesion was associated with the likelihood of having genotype 16/18 (PR for CIN3 = 2.1, 95% CI 1.26-3.54, PR for invasive cancer 3.5, 95% CI 2.11-5.93, ref CIN2) and with genotypes covered for in the 9-valent vaccine (PR for CIN3 = 1.36, 95% CI 1.07-1.73, PR for invasive cancer 1.57, 95% CI 1.22-2.05, ref CIN2) but when adjusting for grade of lesion the PRs associated with HIV-status changed only slightly (Table 6).

## 7 DISCUSSION

The global prevalence of WLWH varies greatly with different regions and it is estimated that around 80% of WLWH are presently living in SSA and 10% in Asia and Pacific<sup>35</sup>. In the same regions we see the highest burden of cervical cancer because of poor access to cervical cancer screening and higher risk when living with HIV<sup>12</sup>.

The study populations of WLWH included in this thesis, although different for all four papers, represent this global disparity well with its domination of migrants, among whom 70% were born in SSA and 10 to 20% in Asia and Pacific. Most previous studies in this field have been based on populations living in the US and lately SSA but studies based in Europe are scarce and studies based on migrants living in Europe even fewer<sup>77,124,154</sup>. Uniquely for all four registry-based studies included in this thesis, we have had access to data regarding country of birth also for included HIV-negative women. This has made it possible for us to control for country of birth that may otherwise cause confounding in a study population based on migrants. Most importantly this has made our results relevant not only to Sweden or Western Europe but also to the global regions where the burden of HIV and cervical cancer is the greatest.

### *Regarding the risk of high-grade cervical lesions and outcome after its treatment*

Earlier studies have found a 2 to 4-fold increased relative risk of CIN2+/CIN3+ in WLWH compared to HNW<sup>10,93,106,124,155</sup>. In **Paper I** we found WLWH to have more than eight times higher risk of CIN3+ than HNW and more than nine times higher risk of CIN2+, which was thus higher than expected and driven by the large difference in risk between migrants living with and without HIV (more on that below). We found only one case of ICC in WLWH during follow-up. Earlier studies have found the absolute risk of invasive cervical cancer in WLWH to be very small when these women are included in cervical cancer screening programs<sup>123,124</sup>.

As expected WLWH were more likely to have treatment failure after treatment of CIN2+ than HNW<sup>104,110,113</sup> (**Paper III**). This was true even when restricting the definition of treatment failure to CIN2+ which contradicts the suggestion that increased treatment failure in WLWH is mainly due to new HPV infections contracted after treatment<sup>104</sup>. We found WLWH to be five times more likely to recur than HNW, although these results should be interpreted with a little caution. There were only 13 cases of recurrence in WLWH and of these a majority where CIN1 so this could in fact, with reference to the discussion above, be due mainly to new HPV-infections rather than true recurrence of neoplastic lesions.

### *Regarding the role of immunosuppression*

Similar to previous studies we found advanced immunosuppression to be associated with both CIN3+ (**Paper I**) and treatment failure (**Paper III**)<sup>10,104,106,110,115,124,156</sup>. In both **Paper I** and **Paper III** the level of immunosuppression as measured by CD4 count at inclusion/treatment of CIN2+ was more robustly associated with outcome (CIN3+/treatment failure) than nadir CD4. When adjusting for CD4 count at inclusion and nadir CD4 in the

same model in **Paper III** the association between level of nadir CD4 and treatment failure decreased even more. This could indicate that present CD4 count is a better predictor of future outcome than the level of CD4 in the past, contrary to what has been stipulated in at least one earlier study<sup>156</sup>. However, as almost all WLWH included had at one point been highly immunosuppressed (about 60% had nadir CD4 <200 in both **Paper I** and **Paper II** and almost 90% had nadir CD4 <350 in both **Paper I** and **Paper II**) we may not have been able to show an association for this variable even if there was one.

#### *Regarding the role of ART*

Even though it has been known for quite some time that HIV-related immunosuppression is associated with increased risk of persistent HPV and the subsequent development of CIN and ICC, the role of ART on these processes has been uncertain. It is only recently that ART has been proven to be associated with reduced prevalence of HR HPV and reduced incidence of HSIL/CIN2+/ICC<sup>78</sup>. To our knowledge, **Paper III** is the first study to find suppressive ART associated with effective treatment of CIN2+. A majority of earlier studies has not had access to HIV-RNA levels and used only self-reported use of ART with the risk of reporting bias<sup>104,110,115</sup>. A small study finding less recurrence (defined as treatment failure in our study) in those on self-reported ART used no measure of effect analysis<sup>157</sup>. One study having access to HIV-RNA levels did not find the HIV-RNA level associated with treatment failure, but unlike ours that study included women treated for CIN1, which is usually self-healing and not recommended to treat<sup>114</sup>.

It is believed that the main benefit of ART, in this setting, is the consequential increase of non-impaired CD4-cells that are needed to clear the HPV-infection. But although it is widely accepted that effective immune control is required to prevent persistent HPV infection, later studies indicate that chronic inflammation and misguided immune responses may also play an important role in HR HPV-induced carcinogenesis, even in HIV-negative individuals<sup>158</sup>.

It has been suggested that in the early stages of HR HPV-induced carcinogenesis the HR HPV-infected cells suppress acute inflammation in the epithelium, while later on HR HPV-transformed cells initiate chronic stromal inflammation, led by an increase of IL-6<sup>158</sup>. Meanwhile, as mentioned earlier, HIV-infection is associated with persistent immune activation and inflammation, illustrated by increased levels of immunologic biomarkers (such as IL-6) that remain increased even after ART is initiated, although at lower levels<sup>28,158-161</sup>. Thus, HIV-induced inflammation, not only reduced CD4 counts, may play an important role in cervical cancer development in WLWH and this role may be diminished although not eradicated with suppressive ART. This could explain why we found WLWH with suppressed ART to still have increased (though not statistically significant) likelihood of treatment failure compared to HNW (OR 1.8: 95% CI 0.8-4.2).

#### *Regarding regional differences*

Unexpectedly, WLWH born in the East region (70% Thai) had more than two times higher risk of CIN3+ compared with WLWH born in Sweden (**Paper I**). If anything one would have expected a higher risk in WLWH born in SSA where the global burden of cervical cancer is the highest but we saw no statistical difference between these women and women born in Sweden. We considered the possibility that WLWH born in East were more

immunosuppressed, due to late HIV-diagnosis (as seen in **Paper II**), which would have increased their risk of CIN3+. However, when adjusting for nadir CD4 and CD4 count at time of inclusion the increased risk remained, although non-significantly for the latter adjustment. One reason could be that sexual history differs depending on region of birth with perhaps increased lifetime HPV exposure in WLWH from the East region.

*Regarding HPV genotypes associated with high-grade cervical neoplasia*

We found HPV 16 to be the most common single infection detected in all women irrespective of HIV-status, although, as expected from earlier studies, the proportion of HPV 16 was lower in WLWH than in HNW (**Paper IV**)<sup>69,162</sup>. Women from SSA have previously been found to have the lowest proportion globally of HPV 16 positivity in all grades of cervical lesions and these interim results support this observation<sup>88</sup>. Meanwhile, a recent US study found a lower HPV 16 prevalence in CIN3+ in African American compared to Caucasian WLWH, indicating a genetic association rather than a geographic one<sup>163</sup>.

It is believed that immune competent individuals control non-HPV 16 better than HPV 16, but that with increasing immunosuppression the proportion of non-HPV 16 genotypes increases among persistent HR HPV infections<sup>83</sup>. The increased prevalence of non-HPV 16 (i.e. less carcinogenic HR HPVs) detected in WLWH diagnosed with CIN2/CIN3 may explain why although cervical cancer is increased in WLWH the absolute risk is not as great as for other virally associated AIDS-defining cancers such as Kaposi sarcoma or non-Hodgkin lymphoma<sup>32,81,164</sup>.

In **Paper IV** we found HPV 35 to be the second most common single infection in WLWH. A majority of WLWH with HPV 35 were born in SSA. This is in line with earlier studies that have shown an increase of HPV 35 detected in all grades of cervical lesions in women born in SSA, compared to other regions of the world, in both HNW and WLWH<sup>69,88</sup>. A recent study, based in SSA, found persistent HPV 35 (and 56) to be the non-vaccine types most commonly associated with incident CIN2+ and a meta-analysis based on WLWH diagnosed with ICC in SSA found HPV 35 to be the fifth most commonly detected genotype<sup>84,165</sup>. These findings suggest that adding HPV 35 to future HPV vaccines should be considered<sup>84</sup>.

The detection of single HPV 18 was lower than expected in all women but especially in WLWH, as previous studies have shown that these women are more likely than HNW to be diagnosed with ICC caused by HPV 18<sup>84,166</sup>. Neither did we see an increase of HPV 45 despite this genotype being previously identified as an important HR HPV type in WLWH in SSA and considering that women born in SSA dominated this study. One reason for this may be the few cases of ICC that were included in this interim analysis. It has been suggested that the carcinogenicity of HPV 18/45 is underestimated in studies based on CIN2/CIN3, rather than ICC, as the increase of HPV 18/45 is mainly seen in the interim between the development of CIN3 to ICC<sup>88</sup>.

Similar to earlier studies we found WLWH to be significantly more prone to have multiple HR HPV infections than HNW<sup>167-169</sup>. The clinical significance of multiple infections is unclear as prospective evidence on this issue is limited<sup>170</sup>. A US study with long-term follow-up did not find multiple HPV infections associated with an increase of CIN3+. In another

study, including only HNW, co-infection with both low and high-risk HPV was even associated with a reduced risk of future invasive disease and slower progression to ICC<sup>86,87</sup>.

### *Regarding HPV vaccination*

It is a concern that among WLWH in **Paper IV** only one quarter had HR HPV genotypes that would be covered by the 2 and 4-valent vaccines and only half had genotypes covered by the 9-valent vaccine. These are lower estimates than what was seen in a recent study based on WLWH with CIN2/3 in Burkina Faso and South Africa (around 40% covered by the 2/4-valent and 80-90% by the 9-valent) and an earlier South African study (42% covered by the 2/4-valent) including women diagnosed with HSIL<sup>101,165</sup>.

A recent meta-analysis, including studies based in SSA indicated that about 60% of WLWH with ICC would have been covered by the 2 and 4-valent vaccines and about 80% by the 9-valent vaccine<sup>84</sup>. If our study had included only ICC we would probably have detected a higher proportion of HPV 16/18<sup>166</sup>. Although the main target for HPV vaccination is ICC, a diagnosis of CIN2/CIN3 will in most cases lead to treatment (usually conization), which is associated with complications such as preterm birth<sup>129</sup>. In addition, as shown in **Paper III**, WLWH are more likely to have treatment failure after treatment of CIN2/CIN3. Given the findings in **Paper IV** it would seem that young PLWH and populations at-risk for HIV, not the least those living in SSA, would benefit more from the 9-valent vaccine than the 2 and 4-valent vaccines. Nevertheless, the extra protection provided by the 9-valent vaccine will not make any difference in LMIC if access is limited by cost and poor infrastructure as in many places today<sup>171</sup>.

### *Regarding cervical cancer screening*

In **Paper I** we found a low adherence to cervical cancer screening in WLWH. To ensure that this was not because of poor registration in NKCx, we reviewed 100 randomly selected medical records from these women and found that nine percent had cervical cytology tests that were not registered in NKCx. This, however, would only partly explain our findings. One reason for low attendance to cervical screening may be that while routines for screening HNW have been very well implemented nationally, the more frequent screening of WLWH is not automatized in the same way and quite often dependent on the awareness of midwives, gynaecologists and HIV-clinicians which may vary between clinics.

Until recently, most high-income countries recommended WLWH to be screened with cervical cytology twice during the first year after HIV-diagnosis followed by annual testing until the age of 64. This has also been the recommendation in Sweden for the time period included in this thesis. With increasing knowledge about HPV and HIV co-infection, several guidelines are now leaning towards having the same/or more similar HPV-based screening programs for women independent of HIV-status<sup>112,125-127</sup>. This may not only increase the adherence to the screening program in WLWH but also decrease costs and reduce risk of harm (such as anxiety after slightly abnormal results and higher risk of pre-term births after conization) caused by possible over diagnosis and overtreatment<sup>129</sup>.

The risk of developing CIN2+ is low in women with a negative HR HPV-test irrespective of HIV status<sup>99</sup>. In Sweden, HPV testing of women includes all HR HPV types, but triaging for

only HPV 16/18 is often recommended, especially in low resource settings<sup>11,122,172</sup>. In **Paper IV** we found that WLWH had a high proportion of non-HPV 16/18 in CIN2+ biopsies, women who would thus not be identified as at risk of developing CIN2+ if screening included only HPV 16/18. Meanwhile WLWH who are HPV-positive with normal cervical cytologies have a very high prevalence (>70%) of non-HPV 16/18 infections<sup>69</sup>. The question therefore remains whether to include HR HPV other than HPV 16/18 in HPV-based cervical cancer screening in WLWH with the risk of reducing the positive predictive value of this test<sup>128</sup>.

#### *Regarding high grade cervical neoplasia as an indicator disease for HIV*

Migrants in EU are at particular risk of late HIV-diagnosis and therefore more likely to present with advanced immunosuppression and disease i.e. have AIDS, at time of HIV-diagnosis. A previous study found that one quarter of newly HIV-diagnosed patients in Sweden had presented for health care with typical HIV/AIDS associated conditions without being HIV-tested<sup>44</sup>. Late HIV-diagnosis is associated with higher mortality and morbidity as well as increased risk of HIV transmission and higher health-care costs<sup>44,173</sup>. Although indicator-guided HIV-testing has been recommended in Europe since 2013 it is not yet widely implemented<sup>44,54,174-176</sup>.

In **Paper II** we found migrant women diagnosed with CIN2+ to have a prevalence of undiagnosed HIV exceeding the threshold estimated to be cost-effective for HIV-testing. It is very important to identify these women who are unaware of their HIV-status so they can initiate ART as soon as possible since they have, as shown in **Paper III**, better outcome after treatment of CIN2+ when on suppressive ART.

It has been discussed over the past years among HIV-clinicians and gynaecologists whether or not to offer HIV-testing to all women in Sweden diagnosed with the indicator disease cervical dysplasia/cancer. There has been hesitancy among gynaecologists to single-out testing of migrants only, while at the same time scepticism as to the cost-benefit of HIV-testing all women. Our results do not support HIV-testing all women diagnosed with CIN2+ in Sweden. However, future updated calculations may find HIV screening cost-effective at a lower prevalence than the presently suggested 0.1%, as the benefits of early ART have been clarified since the latest calculations of cost-effectiveness<sup>26,27,161</sup>.

Even in migrants with no CIN2+, the prevalence of undiagnosed HIV was still quite high (0.08%) with a confidence interval including 0.1% and for women born in SSA and Asia & Pacific with no CIN2+ the prevalence was above 0.1%. Consequently, HIV testing all migrant women with unknown HIV-status at the time of cervical screening/gynecology check-up, irrespective of cervical cytology results, could be considered as these women have very much to gain from getting aware of their HIV-status as soon as possible. An earlier start of ART will, apart from as mentioned earlier reduce mortality, morbidity and transmissibility, more specifically decrease progression of SIL, increase SIL/CIN regression and reduce incidence of ICC<sup>78</sup>.

#### *Limitations of included studies*

Limitations common for all four papers, or of particular interest, are discussed here. For detailed discussions on limitations see individual papers.

Smoking is a known risk factor for the development of cervical cancer, but we were not able to adjust for smoking in these studies since smoking status only recently has been added to the InfCare HIV registry (**Papers I, III, and IV**) and although smoking is supposed to be recorded in medical records, this was most often lacking (**Paper III**)<sup>91</sup>. The number of current or former smokers in the Swedish HIV-cohort is lower compared to other HIV-cohorts. In a recent (yet unpublished) survey including 717 WLWH (24% of WLWH currently living in Sweden) 14% were current smokers and 14% previous smokers<sup>40</sup>. This is in contrast to the number of current smokers among HIV-infected women in the US (50%) and in Denmark (43%)<sup>10,124</sup>. Current smoking was significantly associated with two times higher risk of CIN3+ in a US study, but there was no significant association with former smoking<sup>10</sup>. In Sweden, 9.6% of the female HIV-negative population (16-84 years old) are current smokers<sup>177</sup>. Given the low prevalence of smokers in the Swedish HIV cohort it is not likely that confounding by smoking explains our results.

We did not have access to data regarding sexual history so we could not control for this variable. It is therefore not possible to exclude that the increased risk of CIN3+ seen in WLWH from the East region (**Paper I**) was due to higher lifetime HPV exposure than WLWH from other birth regions. Also we cannot disregard the fact that cases classified as recurrence after treatment of CIN2+ (**Paper III**) may instead be due to new HPV-infections.

For **Paper IV** not all requested cervical blocks were sent from bio banks. In some cases the bio bank refused providing the material due to not-defined reasons, in other cases there was lack of tissue left in the block, or diagnostic slides needed for review were missing. This is not unique to our study and is evidently something that needs improving so that access to bio bank material can continue to be an essential part of Swedish research<sup>63</sup>.

### *Strengths of included studies*

The national registry InfCare HIV is a virtually complete registry that is continually updated and the high quality of data is regularly assessed and confirmed. HIV-clinicians in Sweden have come to rely on InfCare HIV in the daily practice as a tool for explaining lab-results to patients as well as for making clinical decisions and are therefore highly motivated to update patient variables. Lab results (for example HIV-RNA) are automatically included in the registry and we were able to assess the effect of suppressive ART (**Paper III**), instead of as in most previous studies self-reported ART use. We have also been able to control for level of CD4 counts in all papers.

For all four studies we have had access to data from NKCx, a highly functioning nationwide registry of cervical cytology and histopathology. The completeness of the registry is high ( $\geq 90\%$  for both cytology and histology) and based on both screening and colposcopy results irrespective of where the examination has taken place. In other studies in this field cervical screening history has often been self-reported which may lead to recall-bias. For **Paper IV** we included only biopsy specimens (rather than cytology), which enhances the accuracy of the HPV genotyping analysis and reduces the prevalence of multiple infections.



**In summary**, this thesis found that WLWH in Sweden have a larger than expected increased risk of high-grade CIN compared to HNW and that this was driven by a great discrepancy in risk between migrants with and without HIV. In WLWH, we found an unexplained difference in risk of CIN3+ depending on region of birth, remaining after adjusting for immunosuppression. WLWH in our study had more treatment failure and recurrence after treatment of CIN2+ than HNW, but high CD4 counts was protective of treatment failure. For the first time we showed suppressive ART to be associated with effective treatment of CIN2+. WLWH had less proportion of HPV 16 infection in histology-confirmed CIN2+ compared to HNW from the same country of birth and the proportion of WLWH with HR HPV types covered by current HPV vaccines was lower compared to HNW. Further, we found migrants diagnosed with CIN2+ to have a prevalence of undiagnosed HIV above the threshold estimated cost-effective for HIV-testing, indicating that this population should be HIV-tested.

Early HIV diagnosis, access and adherence to ART, HPV vaccination of young people living with HIV and those at high-risk of HIV-infection, and finally access and adherence to cervical cancer screening are all of crucial importance to minimize the incidence of high-grade CIN and its progression to ICC in women living with HIV.

## 8 CONCLUDING REMARKS

In response to specific aims

### I

- The cumulative incidence, incidence rate and risk of CIN3+ were substantially increased in WLWH compared to HNW, which was driven by a great discrepancy in risk between migrants with and without HIV. Among women living with HIV we saw clear differences in risk of CIN3+ depending on region of birth.

### II

- The prevalence of undiagnosed HIV in all women diagnosed with CIN2+ in Sweden **did not** reach the level suggested cost-effective for HIV-testing.
- The prevalence of undiagnosed HIV in migrant women diagnosed with CIN2+ in Sweden **did** reach the level suggested cost-effective for HIV-testing.

### III

- WLWH had poorer outcome after treatment of CIN2+ than HNW.
- Suppressive ART and high CD4 counts were both associated with better outcome after treatment of CIN2+ in WLWH.

### IV

- The proportion of HPV 16 in CIN2+ biopsies was lower in WLWH than in HNW while the prevalence of multiple HR HPV infections was increased in WLWH.
- Only one quarter of WLWH had HR HPV genotypes that would have been covered by the bivalent/quadrivalent HPV vaccine.

## 9 CONSIDERATIONS REGARDING CLINICAL IMPLICATIONS

Immunosuppression was associated with the incidence of CIN2+ and with failure after treatment of CIN2+ in our study. To minimize HIV-related immunosuppression WLWH needs to be identified early, thus enabling an early start of ART.

In Sweden, we need to ensure efficient HIV-testing at time of immigration. An enhancement of the implementation of HIV-indicator guided HIV-testing is needed and migrants diagnosed with CIN2+ should be offered HIV-testing.

Adherence to ART is crucial in PLWH to reduce mortality, co-morbidity and HIV-transmission. Suppressive ART was associated with effective treatment (conization) of CIN2+ in our study. The Swedish InfCare HIV cohort has one of the best HIV treatment results in the world, but globally continuum of care in HIV cohorts is a major issue and needs to be prioritized.

Access and adherence to cervical cancer screening is essential for all women. International and Swedish guidelines now recommend similar or almost similar screening intervals irrespective of HIV status.

WLWH had a reduced proportion of HPV 16 in CIN2+ biopsies compared to HNW in our study. In Sweden, HPV triaging includes all HR HPVs. Efficient, easy-to use and cheap HPV triaging is needed in regions with high prevalence of HIV and which HR HPVs should be included in HPV-triaging in these regions needs to be evaluated.

The 9-valent HPV vaccine seems to be a better choice, than other current HPV vaccines, for the protection of CIN2+ in WLWH. Sweden has just recently decided to exchange the 4-valent for the 9-valent vaccine in the national HPV vaccination program.

## 10 FUTURE PERSPECTIVES

Cervical cancer is one of few cancers that is preventable if HPV vaccination and cervical cancer screening is combined. The main obstacle is to reach high global coverage of HPV vaccination and cervical cancer screening, especially in LMIC. For the prevention of high-grade cervical intraepithelial neoplasia and its progression to cervical cancer in WLWH there are still large gaps of knowledge that needs to be filled. Two research questions of particular interest to me are:

*What is the efficacy of HPV vaccination in PLWH?*

As mentioned earlier, HPV-vaccines have been found to be safe and well tolerated in PLWH (children and adults) and do not alter the CD4 count or HIV viral load of those vaccinated<sup>138-141,178</sup>. The antibody response, however, is significantly lower in girls living with HIV relative to HIV-negative girls, especially when immunosuppressed, but whether this is a clinically relevant decrease remains uncertain<sup>142,144</sup>. Suppressive ART seems to predict a better antibody response<sup>179</sup>. To date there is very little data concerning the efficacy of HPV vaccination in PLWH<sup>145,180</sup>. The only efficacy study published this far found less persistent HPV in vaccinated girls/women living with HIV compared to unvaccinated WLWH<sup>145</sup>. There was no HNW comparison group in this study, which was relatively small (n = 279) and with only two years of follow-up. Large HPV vaccine efficacy studies, with long-term follow-up and with the statistical power to assess the effect of immunosuppression and suppressive ART, are highly needed in PLWH.

*What HR HPV-genotypes are associated with treatment failure after treatment of CIN2+ in WLWH?*

Data is scarce regarding which HR HPV genotypes are associated with HR HPV persistence after treatment of CIN2/CIN3 in WLWH. A Kenyan study (n = 85) with short-term follow-up (six months) found that cryotherapy eliminated three quarters of CIN2/3 but only one fifth of HR HPV infections in WLWH<sup>181</sup>. They did not find persistence of HR HPV associated with CD4 count or time on ART, which may have been due to lack of power. A US study (WLWH n = 170, HNW n = 15) found 67% of WLWH with treatment failure to have persistent HR HPV but they did not specify which genotypes were associated with treatment failure. More data, preferentially based on larger studies, is needed on HR HPV type persistence and its association with treatment failure and recurrence in WLWH treated for CIN2+. This is important for the implementation of evidence-based guidelines for post-treatment follow-up in these women.

## 11 SVENSK SAMMANFATTNING (SWEDISH SUMMARY)

Med tillgång till effektiv hivbehandling har dödligheten i hiv/aids minskat dramatiskt och förväntad livslängd närmar sig den för hivnegativa individer. Dock ser man bland individer som lever med hiv en ökad risk för att utveckla cancer.

Det är välkänt att kvinnor som lever med hiv har en ökad risk för persistenta onkogen infektioner med humant papillomvirus (HPV) och därmed också en ökad risk att utveckla livmoderhalscancer och dess förstadier. Fram tills nu har det varit okänt hur förekomsten ser ut bland kvinnor som lever med hiv i Sverige. Syftet med denna avhandling var att analysera omfattningen av allvarliga förstadier till livmoderhalscancer (CIN2+/CIN3+) bland kvinnor som lever med hiv i Sverige, att bedöma vilka kvinnor som har störst risk för att utveckla CIN2+/CIN3+ och orsaken till detta (arbete I), hur utfallet är efter en behandlingsåtgärd av dessa förstadier (arbete III) samt vilka HPV-genotyper som orsakat dessa förstadier (arbete IV). Vi ville även undersöka om förstadier till livmoderhalscancer är att klassas som en indikatorsjukdom för hiv i Sverige d.v.s. om kvinnor som diagnostiseras med dessa cancerförstadier bör hivtestas (arbete II).

**Arbete I** är en registerstudie där vi inkluderade 893 kvinnor som lever med hiv från det nationella hiv registret (InfCare HIV) och 205 842 hiv-negativa kvinnor från det svenska befolkningsregistret, frekvensmatchade efter födelseår och födelseregion. Studiepopulationen länkades sedan till det Nationella Kvalitetsregistret för Cervixcancerprevention (NKCx). Efter 18 års uppföljning hade hela 13 % av kvinnor som lever med hiv fått mycket allvarliga cellförändringar (CIN3+) jämfört med 2 % för hivnegativa, vilket motsvarar en mer än 8 gånger ökad risk och var relaterat till graden av immunsvikt. Hivinfekterade med östlig födelseregion (dominerade av thailändska kvinnor) hade två gånger högre risk för CIN3+ jämfört med kvinnor som lever med hiv födda i Sverige, en risk som kvarstod efter justering för graden av nedsatt immunförsvar. Deltagandet i regelbunden screening för livmoderhalscancer var lågt för kvinnorna som lever med hiv, oavsett födelseregion.

I **arbete II** utförde vi en registerstudie där vi inkluderade 960 577 kvinnor födda mellan 1940 och 1990, boende i Stockholm eller Göteborg någon gång mellan 1990 och 2014, som hade minst ett registrerat cellprov i NKCx. Efter att ha länkat dessa kvinnor till InfCare HIV och analyserat sannolik tidpunkt för hivinfektion (med en s.k. trajektorisk CD4 modell) kunde vi uppskatta hur många kvinnor som hade en odiagnostiserad hiv vid tidpunkten för diagnos av CIN2+. Vi fann att förekomsten av odiagnostiserad hiv vid CIN2+ diagnos inte nådde den nivå (0.1%) då hivtest rekommenderas när vi tittade på alla kvinnor i studiepopulationen. Däremot hade migranter med konstaterad CIN2+ en förekomst av odiagnostiserad hiv på 0.3% vilket indikerar att denna population bör hivtestas.

**Arbete III** är en registerstudie där vi länkade InfCare HIV med det svenska befolkningsregistret och med NKCx. Vi inkluderade 179 kvinnor som lever med hiv med konstaterat förstadium till livmoderhalscancer (CIN2+) och matchade varje kvinna med två hivnegativa kvinnor, diagnostiserade med CIN2+, från samma födelseland. Kompletterande information hämtades från patientjournaler. Vi fann att efter behandling (konisering) av CIN2+ hade kvinnor som lever med hiv en mer än tre gånger ökad risk för utebliven läkning

och en fem gånger ökad risk för återfall. De kvinnor som lever med hiv som i vår studie hade en välfungerande hivbehandling vid konisering av CIN2+ hade en bättre chans till utläkning, vilket denna studie är först med att påvisa. Ett bra immunförsvar var även det kopplat till bättre chans till utläkning.

I **arbete IV** studerade vi samma kvinnor som i arbete III men nu för att se om de HPV-genotyper som orsakat CIN2+ hos dessa kvinnor skiljde sig åt beroende på om kvinnan lever med hiv eller ej. Vi fann att den HPV genotyp (16) som oftast orsakar livmoderhalscancer var mindre vanlig bland kvinnor som lever med hiv än bland de hivnegativa kvinnorna. Vi fann också att endast en fjärde del av kvinnor som lever med hiv jämfört med hälften av de hivnegativa kvinnorna hade onkogen genotyper som är inkluderade i det 2-valenta/4-valenta HPV vaccinet. Det 9-valenta HPV vaccinet tycks vara ett bättre val för ungdomar/kvinnor som lever med hiv och i populationer med hög risk att smittas med hiv.

**Sammanfattningsvis** visar denna avhandling att kvinnor som lever med hiv i Sverige har en markant ökad risk för allvarliga livmoderhalsförändringar jämfört med hivnegativa och att födelseregion och grad av immunsvikt tycks påverka risken. Migranter med konstaterat allvarliga livmoderhalsförändringar bör hivtestas då denna population har en ökad förekomst av odiagnostiserad hiv. Kvinnor som lever med hiv har en ökad risk för att inte läka ut allvarliga livmoderhalsförändringar efter att ha genomgått behandling (vanligtvis konisering) av densamma men bättre chans till utläkning om de har en välfungerande hivbehandling. Det är av stor vikt att kvinnor som lever med hiv deltar i screening för livmoderhalscancer och ungdomar som lever med hiv bör HPV vaccineras. Det 9-valenta HPV vaccinet tycks vara ett bättre val än det 2-valenta/4-valenta HPV vaccinet för ungdomar/kvinnor som lever med hiv.

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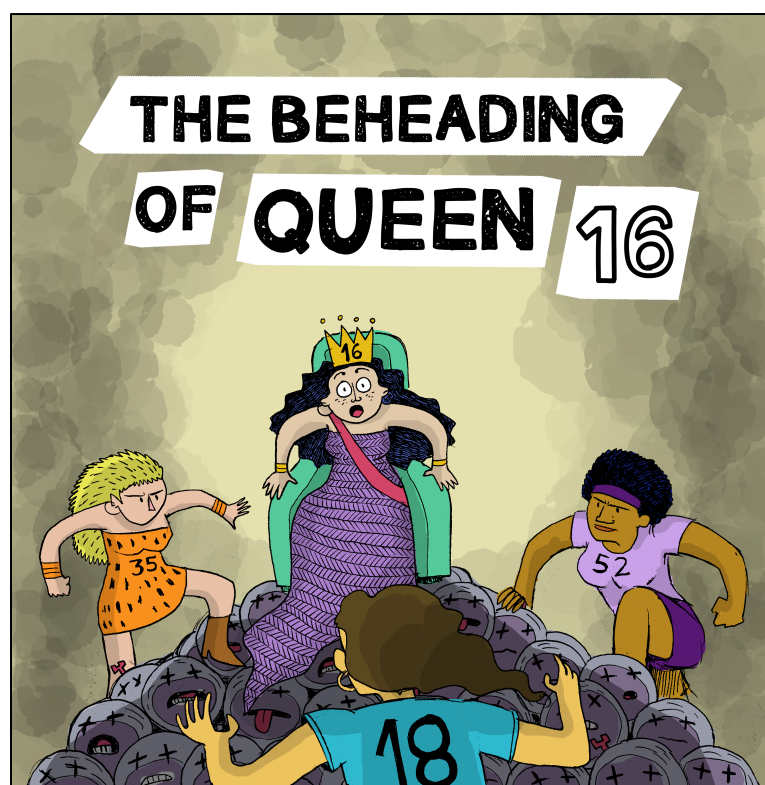
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**Figure 17. The rule of Queen HPV 16 is threatened by other genotypes.**  
Cartoon by @Pedromics.

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